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THE BAR "LOCUS" AND THE v^+ REACTION IN DROSOPHILA MELANOGASTER

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Communicated February 5, 1938

Beadle and Ephrussi,[2] using the transplantation technique (Ephrussi and Beadle[3]), found that the formation of wild type eye pigmentation is dependent upon the presence of at least two substances, the so-called vermilion$^+$ (v^+) and cinnabar$^+$ (cn^+) substances. It was shown that while vermilion eyed flies were completely lacking in both these substances,[1] wild type flies not only possessed them but could supply them to implanted eyes. Thus, the only source of the v^+ and the cn^+ substances for an eye disc implanted into a vermilion host is the eye disc itself, while an eye disc implanted into a wild type host may secure these substances from the host as well. Beadle and Ephrussi reported that imaginal discs of the Bar eye of *Drosophila melanogaster*, when implanted into vermilion hosts, developed vermilion rather than + pigmentation, whereas imaginal discs of heterozygous Bar eyes developed + pigmentation when implanted into vermilion hosts. They offered further evidence to show that the failure of the homozygous Bar eye to form + pigmentation is not due to its smaller size. Beadle and Ephrussi suggested that the failure of the Bar eye to develop + pigmentation may be due to a retardation of certain reactions in the eye relative to the states of certain developmental reactions in other organs, and that this retardation is due to a recessive effect of Bar.

The experiments reported below are the first of a series designed to test further the above hypothesis in order to investigate this phase of the "Bar reaction." It is hoped that as a result some further light may be shed on the Bar reaction as a whole as well as on the method of pigment formation in the eye.

By means of the Ephrussi and Beadle transplantation technique the imaginal discs of the following Bar "alleles" (with wild type eye color) and their heterozygotes with + were implanted into vermilion hosts (with wild type eye size): B, BB, B^1, B^1B^1 and B^4 (Bar, double Bar, infra Bar, double infra Bar and Bar^4, respectively). In addition, eyes containing

B in combination with $m(B)$, a semi-dominant inhibitor of B located in the second chromosome (Steinberg unpublished), were implanted into vermilion hosts. Both donors and hosts were larvae about to pupate. All experiments were conducted at 25 ± 1°C. In all except two cases the controls were implants of the same Bar genotype into wild type hosts (normal eye size and pigmentation), and the same Bar genotype in combination with vermilion implanted into vermilion hosts. The two cases in which this was not true are B^sB^s/B^sB^s and B/B; $m(B)/m(B)$ (modifiedBar). The controls for B^sB^s/B^sB^s were $BB/+$ into $+$ and vBB/v into vermilion. Those for B/B; $m(B)/m(B)$ were $B^sB^s/+$ into $+$ and vB^sB^s/v into vermilion. This was done because the facet numbers of B^sB^s/B^sB^s and $BB/+$ and of B/B; $m(B)/m(B)$ and $B^sB^s/+$ are alike or very nearly so. In all cases judgments were based on direct comparison of test and control implants; for example, Bar eyes implanted into vermilion hosts were compared at the same time to Bar eyes implanted into wild type hosts and to vermilion Bar eyes implanted into vermilion hosts (cf. table 1). Inasmuch as neither the sex of the donor nor that of the host had any effect on the phenotype of the implant, sex is not considered in the following report.

Before the data are discussed as a whole, it is necessary to call attention to the pigmentation of the homozygous and heterozygous Bar eyes, when implanted into vermilion hosts. As stated above, Beadle and Ephrussi[2] reported that B/B developed vermilion pigmentation, while $B/+$ developed $+$ pigmentation when implanted into vermilion hosts. Reference to table 1 will show that in our experiments B implanted into vermilion hosts was slightly but definitely darker than vB implanted into vermilion hosts, and that $B/+$ implanted into vermilion hosts was considerably lighter than $B/+$ implanted into $+$ hosts. Since our experiments were conducted at the same temperature and with larvae of the same age as those used by Beadle and Ephrussi, we are at a loss to account for the differences between our results and theirs. The data in table 1 show that B anlagen implanted into vermilion hosts develop some $+$ pigmentation and that $B/+$ implanted into vermilion hosts is intermediate in pigmentation; it follows therefore that the effect of the Bar "locus" on the pigment reaction is not a simple recessive effect as Beadle and Ephrussi suggest.

The facet numbers given in table 1 are, in all except two cases (modified Bar and Bar[4]), taken from Sturtevant[4] and are used as a standard to indicate the relative sizes of the eyes of the various donors. The Bar "alleles," when implanted into wild eyed hosts, retain the same size sequence as is shown by the non-implanted eyes. In this respect it is of interest to note that the imaginal discs of the various Bar "alleles," as seen in late third instar larvae, also fall into the same size sequence. The data point to some direct correlation between facet number and depth of pigmentation.

The correlation is, however, subject to two exceptions: 1. $B^iB^i/+$ which is approximately one-half as large as B^i/B^i is not only not lighter than B^4/B^i but is considerably darker, and 2. B^4, which is exceeded in facet number only by $B^i/+$ and $+$, is lighter than $B^iB^i/+$, $B/+$ and B/B; $m(B)/m(B)$, all of which are less than one-half as large as B^4.

These discrepancies indicate that, although both the reduction in facet number and the partial or complete loss (cf. BB/BB table 1) of the ability to form the v^+ substance are due to changes at the Bar "locus," they are probably a result of two separate chains of reactions; i.e., the reaction chain resulting in the reduction in facet number is not the one involved in the pigment effect. If this is true, it should be possible to affect one of the reaction systems without affecting the other. It should be possible, for

TABLE 1

DATA ON THE VARIOUS EYE IMPLANTS

The hosts in all cases were v. v pigmentation = 1, $+$ pigmentation = 5 on an arbitrary scale. Values in brackets are those reported by Beadle and Ephrussi[1] in comparable experiments. In all except two cases ($B^iB^i/+$ and $B/B; m(B)/m(B)$, see text) the controls were implants of the same Bar genotype into wild type hosts (wild type controls) and the same Bar genotype in combination with vermilion implanted into vermilion hosts (vermilion controls).

IMPLANT GENOTYPE	FACET NUMBER*	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
BB/BB	25	10	1.0
B^iB^i/B^iB^i	38	12	1.5−
$BB/+$	45	15	1.5−
B/B	68	18	1.5− (1.0)
$B^iB^i/+$	200	14	3.0
$B/B; m(B)/m(B)$	200	9	3.0
B^i/B^i	348	13	2.0
$B/+$	358	7	3.0 (5.0)
B^4/B^4	?	8	2.5
$B^i/+$	716	19	5.0−
$+/+$	779	13	5.0−

* See text.

example, to cause a change in facet number resulting from a given genotype without causing any change in the pigment reaction and vice versa. Experiments are under way to test this hypothesis. An alternative but not as probable an explanation of the data is that B^4/B^4 and $B^iB^i/+$ may involve a qualitatively new situation as compared with the other genotypes, thus producing the observed discrepancy.

During the course of these experiments nineteen fragmented eyes were recovered from transplanations involving $+^vB^{\text{"alleles"}}$ eye discs implanted into vermilion hosts. Table 2 lists these cases showing the total number of fragmented eyes recovered and the number of these which did not develop

as much pigment as did the unfragmented eyes.[5] In no case was a fragmented eye darker than an unfragmented eye. In the cases of $BB/+$ and B/B it is difficult to be certain that the fragments really were not lighter in pigmentation than the entire eyes, since the latter were already very close to vermilion. Consequently, they are not included in the following discussion.

Five of the fragmented eyes failed to develop the same pigmentation as the intact eyes. The fragmented eye recovered in the $B^i/+$ experiment was broken into four pieces. The two largest developed as much pigment as did the unfragmented eyes, the smallest fragment was lightest (vermilion or very close to vermilion), and the fourth fragment was inter-

TABLE 2

DATA ON FRAGMENTED EYES RECOVERED FROM EXPERIMENTS IN WHICH THE DONORS WERE $+vB^{\text{"alleles"}}$ AND THE HOSTS WERE v^5

IMPLANT GENOTYPE	FACET NO.*	PHENOTYPE OF INTACT EYE	NUMBER OF FRAGMENTED EYES	NUMBER FAILING TO DEVELOP SAME PIGMENT AS INTACT EYES*
$BB/+$	45	1.5−	1	0
B/B	68	1.5−	2	0
$B/B;m(B)/m(B)$	200	3.0	1	1
$B^iB^i/+$	200	3.0	4	1
$B/+$	358	3.0	1	0
$B^i/+$	716	5.0−	6	1
$+/+$	779	5.0−	4	2

* See text.

mediate in both size and pigmentation. Of the two fragmented eyes recovered in the $+/+$ experiment one consisted of six pieces and the other, of four. Nine of these ranged in size from smaller than B to B, all being close to vermilion in pigmentation. One fragment about the size of $B^iB^i/+$ was only slightly lighter than the unfragmented eyes. In the remaining two cases ($B^iB^i/+$ and $B/B;m(B)/m(B)$) the eyes were each broken into two pieces. In both cases the smaller fragment was vermilion. the larger the same as the intact eyes. In all of the above cases the smaller fragments were as light as or lighter than the larger ones.

Of the eleven fragmented eyes which developed the same amount of pigment as did the intact eyes, those arising in the $B^iB^i/+$, $B^i/+$ and $+/+$ experiments are of particular interest. Out of a total of fourteen fragmented eyes recovered in these three experiments, four failed to develop the "normal" pigmentation (one in each of the two former, two in the latter). In the ten remaining eyes many of the fragments, which did develop as much pigment as the unfragmented eyes, were as small as or smaller than those which failed to do so. This makes it seem highly

probable that the ability or lack of ability of a fragment to form the v^+ substance involves at least two factors, namely, the size of the fragment itself and the portion of the disc from which the fragment arises. Experiments are planned in which both the size and origin of the fragment will be controlled.

Summary.—The various Bar "alleles" and their heterozygotes with + were implanted into vermilion.

There is an apparent but not entirely consistent correlation between facet number and ability to form the v^+ substance, i.e., smaller eyes show less + pigmentation.

It is suggested that the facet reducing reaction system is not the same as the reaction system affecting pigment formation.

Evidence is offered which indicates that the ability of a fragment to form the v^+ substance is a function of the size of the fragment and of the portion of the disc from which the fragment arises.

The authors wish to acknowledge their indebtedness to Dr. Boris Ephrussi of the Institut de Biologie Physico-Chimique, with whom the senior author carried on preliminary work on these experiments at the Carnegie Institute of Washington, in Cold Spring Harbor, during the Summer of 1937, and to Professor L. C. Dunn of Columbia University for his many helpful suggestions.

[1] Beadle, G. W., *Genetics*, **22**, 587–611 (1937).

[2] Beadle, G. W., and Ephrussi, Boris, *Ibid.*, **21**, 225–247 (1936).

[3] Ephrussi, Boris, and Beadle, G. W., *Amer. Nat.*, **70**, 218–225 (1936).

[4] Sturtevant, A. H., *Genetics*, **10**, 117–147 (1925).

[5] In experiments involving $+^{v}B^{(\text{alleles})}$ eye discs implanted into + hosts 14 fragmented eyes were recovered. In all 14 cases the fragmented eyes developed the same pigmentation as the intact eyes.

SEX REACTION TYPES AND THEIR INTERRELATIONS IN PARAMECIUM BURSARIA. I

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Communicated February 1, 1938

The discovery by Sonneborn[1] of two sex reaction types in a race of *Paramecium aurelia* opened a new chapter in the genetics of the Ciliata. The present study shows that similar reaction types occur in *Paramecium bursaria*, but that in number and interrelationships they differ greatly from the situation in the race of *Paramecium aurelia* studied by Sonneborn and by Kimball.[2]

In the work on the cytology of conjugation in *Paramecium bursaria*, in this laboratory, Dr. T. T. Chen observed that conjugating pairs could be obtained from mixtures of two of his cultures, but not from either culture separately. This formed the starting point of the present investigation. Two clones, designated *l* and *m* were obtained from the cultures of Chen, and the work was extended to many other stocks. The clones *l* and *m* had been collected from a pond at Alexandria, Virginia.

The mating reaction that occurs when two appropriate clones of *Paramecium bursaria* are mixed—such clones as *l* and *m*—is of the rapid agglutinative character described by Sonneborn[1] in *Paramecium aurelia*, and by Moewus[3] for various flagellates. Within a few seconds after mixture the individuals have clotted together in small groups containing two to a dozen or more individuals. In strongly marked reactions these groups quickly coalesce into masses that contain hundreds of individuals and are visible to the naked eye. The animals remain clotted for a half hour to two hours; in the latter part of that period the clots begin to break up, and it is now found that many of the component individuals have become united in pairs. After about two hours only united pairs and single individuals may be found in the mixture.

The pairs are formed from one member of each of the two clones that were mixed. This is readily demonstrated by removal of the green color from individuals of one of the two clones. The usual strong green color of *Paramecium bursaria* is due to the presence in the body of many cells of a green alga. The color can be almost completely removed by inducing the animals to multiply rapidly in a rich nutritive medium, such as the algal-bacterial medium described by Raffel.[4] The contained algae multiply much less rapidly than the Paramecia, so that the later generations contain but a few scattering alga cells which hardly color them at all. In this condition *Paramecium bursaria* is practically as colorless as *Paramecium aurelia*. When one of the two clones is thus made white, the pairs in the mixture are

found to consist always of one green individual and one white one. In the large clots also, green individuals are always in contact with white ones.

The reaction between two clones belonging to diverse reaction types occurs whenever the two clones are mixed, provided they are in the proper physiological condition. There is a period after conjugation, varying in length in different clones, in which the individuals will not mate. Further, mating does not occur when the individuals have recently been transferred to a rich nutritive medium and have grown very large and are rapidly dividing. The most favorable conditions for the mating reaction are furnished when, after such a period of rapid growth and division, the nutritive medium becomes poor and the animals are becoming thin and ceasing to divide. At such periods, if two clones of diverse type are mixed the clotting is strongly marked and many pairs are formed.

Different clones show marked differences in the readiness with which they are brought into the condition for clotting and conjugation. Some are refractory and can be induced to react only by bringing about the exact degree of nutritive decline that is most favorable to the mating reaction. Others react readily under all conditions except those of extreme plumpness and rapid fission resulting from rich nutritive conditions. The reaction occurs, however, only at certain periods of the day, diverse in different stocks, but excluding in all cases the evening hours, so far as observed. (These matters are to be dealt with separately.)

The clotting reaction above described occurs only when two clones of diverse sex reaction type are mixed. But in certain clones, perhaps in all, isolated pairing may occur between members of the same clone, if the clone is left for long periods in a condition of declining nutritive strength. If, for example, great numbers of individuals of a single clone are left for several days in a watch glass with a small amount of water, after some days isolated pairs may sometimes be found among them. The formation of such pairs is not accompanied by clotting, and is so rare that close study of a clone for months may yield no examples. Such exceptional pairs are left out of account here; they form no part of the usual picture of pairing. Their genetic consequences will be dealt with elsewhere.

In such clones as *l* and *m* we thus find two sex reaction types that are seemingly comparable to the plus and minus sex types of Chlamydomonas and other flagellates as described by Moewus,[3] and to types I and II of Paramecium aurelia (Sonneborn 1).

The matter was investigated further (1) by allowing *l* and *m* to conjugate together, producing many exconjugant clones, the sex reactions of which were examined, and (2) by collecting from natural habitats many stocks and studying their sex reactions. Results of the former line of work are presented in this paper.

Sex Reaction Types of Clones Descended from Exconjugants of Type l by Type m.—A large number of pairs were obtained by matings between the clones *l* and *m*, representing the two reaction types. From the exconjugants of these pairs clones were obtained, and the sex type of each was tested by examining its reactions against the two parental types. The design was to obtain if possible inheritance ratios for the two diverse types.

This purpose meets a serious obstacle in the great mortality among the exconjugant lines. Most of the exconjugants or their descendants die. This occurs even in the most favorable nutritive media and other conditions in which non-conjugants flourish, none dying. A large proportion of the exconjugants die without dividing. Others divide, but produce weak offspring, often small and abnormal, which die after a few generations. In the experiments, 142 pairs were isolated from $l \times m$; these should yield 284 exconjugants, and as two lines were to be cultivated from each exconjugant, there were potentially 568 lines of descent. But out of these, only 26 formed clones that continued to multiply and so could be tested; thus but 4.6 per cent. And these included representatives of but 11 of the 142 pairs.

With so high a mortality the determination of inheritance ratios for the sex reaction types is not possible, but study of the 26 descendant clones nevertheless furnishes important information as to the sex reaction types and their nature.

The exconjugant lines were designated as follows. To each pair was given a number, and the two members of a pair were called *a* and *b*. From each member two lines of descent, derived from the first division of the exconjugant, were isolated; these were designated 1 and 2. Thus the pair 67 furnished the four lines of descent 67*a*1 and *a*2, 67*b*1 and *b*2. The 26 exconjugant lines were tested by mixing samples of them respectively with *l* and with *m*, to determine the sex type. As was expected, some reacted only with *l*, others only with *m*.

But the clone 67*a*1 was found to form pairs with both *l* and *m*, and on examination this was found to be the case with all the 4 clones descended from pair 67—*a*1, *a*2, *b*1 and *b*2. They reacted strongly, forming immediately large clots, and numerous later pairs, equally with *l* and with *m*.

It was at first suspected that such clones must have become differentiated into the two sex types, as *Paramecium aurelia* differentiates into two sex types at endomixis, according to Kimball.[2] But examination showed that this is not the case. Any single individual of 67*a*1 (or the other clones of 67) reacts with equal readiness with either *l* or *m*.

This is discovered as follows. A single individual of 67*a*1 is introduced with capillary pipette into a drop containing individuals of clone *l* only. The first individual of *l* with which it comes in contact clings to it and initiates the pairing reaction. While in this conidtion, before firm union, the pair are removed to a drop containing only individuals of *m*. In the transfer

the two become separated, since they have not yet firmly united. Each then provokes a strong reaction with *m;* in a few seconds two small clots are formed. In one of these individuals of *m* are clinging to *l*, in the other to 67*a*1. It is demonstrated that the single individual of 67*a*1 reacts with equal readiness with type *l* or type *m*.

		1				2									3														4			
		m	C	23b1	23b2	l	B	3a1	5b1	15b1	28b1	107a1	107a2	88a1	5b2	67a1	67a2	67b1	67b2	88a2	88b1	88b2	97a1	97a2	97b1	97b2	169a2	1b1	169a1	169b1	169b2
1	m	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	23b1	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	23b2	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	l	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3a1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5b1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	15b1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	28b1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	107a1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	107a2	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	88a1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	5b2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	67a1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	67a2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	67b1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	67b2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	88a2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	88b1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
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	97b1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	97b2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	169a2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
4	1b1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	169a1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	169b1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	169b2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−

TABLE 1

PARAMECIUM BURSARIA

Clones of the $l - m$ group, including 26 clones derived from exconjugants of $l \times m$. Classified table of matings, showing the four reaction types.

\+ Form clots and pairs when mixed.

− Do not clot and pair.

Also, if a single individual of 67*a*1 is allowed to multiply, and some of its descendants are tested with *l*, others with *m*, they are found to react immediately with both.

Thus we have, besides *l* and *m*, a third reaction type, which unites with both *l* and *m*. We are not dealing with simply two reaction types, as was at first assumed.

In the course of the tests a fourth reaction type was found, exemplified by the clone 1*b*1. This forms clots and pairs, not only with *l* and with *m*, but also with 67*a*1 and other members of its type.

To determine the exact situation, each of the 26 clones descended from *l* and *m* was tested with every other, as well as with the parental clones *l* and *m*. In addition, two other clones, *B* and *C*, were found to interact with members of this group; these were included in the tests. The testing of each of the 30 clones with each other one involves $\frac{1}{2}$ (30 × 29) or 435 tests. In each test, 50 to 100 or more individuals of each of the two clones to be tested are placed together in 2 or 3 drops of water and observed for clotting and pairing.

The results of these 435 tests are shown in table 1. As the table shows, the 30 clones (26 derived from $l \times m$) fall into 4 diverse sex reaction types; there are indeed 4 types among the descendants of the two types $l \times m$.

The first of these is the *m* type, containing but four clones, including two descended from $l \times m$.

The second, the *l* type, includes nine clones; among them seven derived from the union of *l* and *m*.

The third, the 67*a*1 type, contains 13 clones, all derived from the union of *l* and *m*.

The fourth, the 1*b*1 type, contains 4 clones descended from $l \times m$. Thus the 26 exconjugant clones derived from the union of *l* and *m* fall into four reaction types in the proportions 2:7:13:4.

The four types have the peculiar relation to each other that the members of each one clot and conjugate with the members of all the other three. The relations for the four types are shown in table 2, in which the plus sign indicates clotting and pairing in the mixture indicated.

TABLE 2

THE FOUR SEX REACTION TYPES AND THEIR INTERACTIONS, IN THE *l* – *m* GROUP

	l	*m*	67*a*1	1*b*1
l	−	+	+	+
m	+	−	+	+
67*a*1	+	+	−	+
1*b*1	+	+	+	−

Certain points with regard to inheritance may be gleaned from table 1. From the union of two parent types, *l* and *m*, we obtain 4 types; two of which (*m* and 1*b*1) are less abundant than the other two In some cases all four clones descended from a pair are of the same reaction type; this is the case in pairs 67 and 97. In other cases lines derived from the same pair,

and even from the same exconjugant of a pair, belong to different reaction types. This is the case for example with 5*b*1 and 5*b*2, with 88*a*1 and 88*a*2, with 169*a*1 and 169*a*2. A segregation of the sex types thus occurs in some cases at the first division after conjugation, as is true in *P. aurelia* (Sonneborn[1]).

The clones produced from the exconjugants of $l \times m$ differ in many ways besides the differences in their sex types. There are characteristic differences in size, in form, in vigor and particularly in their readiness to react sexually. Some, like the parental clones, react quickly under almost any condition. In others, specially favorable conditions must be supplied, of the nature already mentioned, in order to make possible the characteristic reactions when they are placed with individuals of another type.

A discussion of the relation of these facts to the problem of sex differentiation is reserved until the sex reaction types of numerous stocks collected in nature have been presented.

[1] Sonneborn, T. M., *Proc. Nat. Acad. Sci.*, **23**, 378–385 (1937).
[2] Kimball, R. F., *Ibid.*, **23**, 469–474 (1937).
[3] Moewus, F., *Arch. f. Protistenk*, **80**, 467–526 (1933).
[4] Raffel, D., *Biol. Bull.*, **58**, 293–312 (1931).

SEX REACTION TYPES AND THEIR INTERRELATIONS IN PARAMECIUM BURSARIA. II. CLONES COLLECTED FROM NATURAL HABITATS

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Twenty-seven clones of *Paramecium bursaria* were collected from diverse localities: four from near Alexandria, Virginia, twenty-three from the neighborhood of Baltimore, Maryland. After allowing the single individuals to multiply in the laboratory, the sex type of each clone was tested, originally by mixing with individuals of the two first-discovered types *l* and *m*, described in the foregoing paper.

The four Virginia clones, *l* and *m*, *B* and *C*, which all came from the same pond, were found to form but two reaction types, *B* being of the same type as *l*, *C* of the same type as *m*.

But unexpectedly, the clones from the region about Baltimore did not react with any of the Virginia types. As shown in the foregoing paper, two additional reaction types were developed from the two that originally occurred in the Virginia group. None of the 23 Maryland clones reacted with

any of the four reaction types of the Virginia group. In mixtures of any Maryland clone with any Virginia clone, there is no clotting or pairing.

The twenty-three Maryland clones were further tested by making mixtures of each clone with each other one, giving rise thus to 253 mixtures. Five of the Maryland clones did not react with any others. Repeated mixtures of these with each of the other clones were made, but no clotting or pairing occurred under any conditions.

		5						6						7				8	9
		AR	Ff	Fg	Fj	Fm	S	Fa	Fb	j	T	U	V	Fd	Fh	Fk	Fp	Fl	Fo
5	AR	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
	Ff	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
	Fg	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
	Fj	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
	Fm	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
	S	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
6	Fa	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
	Fb	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
	j	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
	T	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
	U	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
	V	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
7	Fd	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
	Fh	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
	Fk	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
	Fp	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
8	Fl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
9	Fo	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−

TABLE 3

PARAMECIUM BURSARIA

Clones from natural habitats near Baltimore, Maryland, showing the five diverse sex reaction types (numbered 5 to 9).

+ Form clots and pairs when mixed.

− Do not clot and pair.

The other 18 Maryland clones reacted in some of the mixtures. The reactions fall into a system which is shown in table 3. Every clone conjugates with the members of certain other clones, and refuses to conjugate with those of certain others.

As table 3 shows, the 18 Maryland clones fall into five diverse reaction types, in addition to the four reaction types shown by the clones of the Virginia group. The Maryland types are numbered 5 to 9 in table 3. The reaction type 5 includes 6 clones, the members of which do not conjugate together, but do conjugate with the members of the four other types of the Maryland group. Type 6 again contains six clones, which do not interconjugate, but do conjugate with the members of the five other types of this group. The seventh type consists of four clones, while types 8 and 9 consist each of a single clone. Members of each type clot and conjugate with the members of every other type, but not with members of their own type.

	1	2	3	4	5	6	7	8	9
1	−	+	+	+	−	−	−	−	−
2	+	−	+	+	−	−	−	−	−
3	+	+	−	+	−	−	−	−	−
4	+	+	+	−	−	−	−	−	−
5	−	−	−	−	−	+	+	+	+
6	−	−	−	−	+	−	+	+	+
7	−	−	−	−	+	+	−	+	+
8	−	−	−	−	+	+	+	−	+
9	−	−	−	−	+	+	+	+	−

TABLE 4

PARAMECIUM BURSARIA

The nine sex reaction types thus far discovered, with their interactions, showing the two groups. Types 1 to 4 form the first or Virginia group, types 5 to 9 the second or Maryland group.

+ Form clots and pairs when mixed.

− Do not clot or pair.

Thus the nine diverse sex reaction types so far observed in *Paramecium bursaria* fall into two independent groups, the members of which do not conjugate with each other. The nine diverse types and their interrelations, with the fact that they fall into two groups, are shown in table 4.

The relations of the nine diverse reaction types to the localities from which they come are of interest. The four reaction types (numbers 1 to 4) which constitute the Virginia group all come from the same pond at Alexandria, Virginia, or are derived by descent from those that came from this pond. The five remaining types, constituting the Maryland group, come from two ponds near Baltimore. Type 5 consists of six clones from a small pond ("Falls Road Pond") a few miles north of Baltimore. Other clones from this pond are found in the other four types, 6 to 9. Type 6 contains four clones (*j*, *T*, *U*, *V*) from the central pond of the Botanical Garden of Johns Hopkins University, and in addition two clones from the Falls Road Pond. The remaining three reaction types are all composed of clones from the Falls Road Pond. Thus the Falls Road Pond contained members of all the five reaction types of the Maryland group.

In view of the two diverse reaction groups formed respectively by the clones from Virginia on the one hand, by those from Maryland on the other, examination of clones from other localities will be of interest; the author

expects to study such, as they can be obtained. The conditions thus far found suggest that there may be many local races, differing in sex reaction type, and not reacting with races from distant localities.

A deeper understanding of these types must wait upon a study, with adequate numbers, of their inheritance in crosses and in self-fertilization. Such a study is in progress.

What is the relation of the findings in this and the preceding paper to the differentiation of sexes? In contradistinction to the flagellates investigated by Moewus, such ciliates as Paramecium are diploid and each of the two mates furnishes a migratory pronucleus and a stationary pronucleus, the former playing the part of a male gamete, the latter the part of a female gamete. There is thus a reciprocal fertilization, and each of the two mates gives rise to a line of descendants. The two mates are thus equivalent in these respects; each is comparable to a hermaphrodite rather than to a male or female. Nevertheless the two mates are differentiated in relation to the mating reaction, and the studies thus far made point to an interesting series of steps leading toward the development of two definite types comparable to two sexes. The observations on *Paramecium bursaria* here set forth suggest nothing of the sort. They show the existence of many diverse types, the members of any one of which do not conjugate together (under ordinary conditions), but do conjugate with members of various other types. But in *Paramecium aurelia*, as shown by Sonneborn and by Kimball, in a given race there are two and only two reaction types, and any single individual may, by endomixis, produce these two but no others. Yet each type still plays in other respects the part of a hermaphrodite. In the Vorticellidae the differentiation has gone farther; the two types are morphologically diverse, and one plays essentially the rôle of a female, the other that of a male. Seemingly two definite sexes have arisen by gradual specialization among an originally large number of diverse reaction types.

THE RÔLE OF THE MELANOPHORE-DISPERSING HORMONE OF THE PITUITARY IN THE COLOR CHANGES OF THE CATFISH

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Until recent years, color changes in the teleosts were thought to be solely under nervous control. It is the aim of this preliminary paper to report experiments which indicate that in the chromatic behavior of the catfish, *Ameiurus nebulosus*, the secretion of the intermediate portion of the pituitary is the most significant factor in the production of the dark phase of body coloration.

Catfishes between 6 and 8 inches long were collected from nature and used soon after their arrival at the laboratory. All experiments were carried out at 12°C. in slowly running water. Stock animals were kept under identical conditions. Standard black or white backgrounds illuminated by two 100-watt frosted Mazda lamps placed a foot apart and 12 to 15 inches above the aquaria were used.

Every "melanophore diameter" in this paper represents an average of the measurements of at least 25 consecutive dermal melanophores from corresponding areas along the middle ray of the tail fin.

Observations.—A. Background Adaptation of Hypophysectomized Catfishes. Fifty-four hypophysectomized catfishes were used to study the rate of the disappearance of the melanophore-dispersing principle from the circulation of the animal. Immediately after the operation these animals were divided into three groups and placed with unoperated controls on black, intermediate and white backgrounds, respectively. Observations which were frequently recorded may be summarized as follows:

1. The responses of normal and hypophysectomized animals to a *white* background were identical. Both groups paled at a normal rate (Parker '34) and remained in that state as long as the same experimental conditions were maintained (10 days).

2. Freshly hypophysectomized and control catfishes adapted equally well to an *intermediate* background. During the first 8 or 10 hours following the operation the experimental animals were indistinguishable from controls. After that, however, the hypophysectomized fishes showed gradual paling which seemed to reach an end-point about 3 days after the operation. Although the pallor of the experimental animals made them distinguishable from the normal ones they were not much lighter. The melanophores of controls in this experiment measured 85 to 100 μ, while those of the paler hypophysectomized fishes were about 70 μ in diameter.

3. Freshly hypophysectomized catfishes darkened on a *black* back-

ground as effectively as controls but after 10 to 12 hours pallor gradually set in reaching its maximum in 80 to 90 hours. Subsequently the operated fishes exhibited a pronounced contrast to normal black-adapted animals. The melanophore diameter of such black-adapted animals was 125 to 130 μ. The operated black-adapted fishes possessed melanophores about 70 μ in diameter, a size presenting comparative pallor but still 20 to 25 μ larger than completely white-adapted melanophores, which average 45 to 50 μ.

Any of the experimental animals described in paragraphs 2 and 3 will lighten when placed on a white background so as to become indistinguishable from white-adapted controls.

B. Tests for the Presence of the Dispersing Principle in the Blood of Recently Hypophysectomized Catfishes. Because the above experiments suggested that a functional amount of dispersing principle apparently remains in the circulation for 3 or 4 days after hypophysectomy, samples of blood (centrifuged free from cellular content) were taken from catfishes at regular intervals during this time. All of the fluid obtainable from a single catfish was tested by subcutaneous injections into a black-adapted catfish which had been hypophysectomized 10 days. Although this is a crude and rather insensitive test, it indicated that at sixteen hours after hypophysectomy there is present in the circulation sufficient dispersing substance to darken a test animal markedly.

If adrenalin (just enough to produce pallor in a black fish, Bray '18) is injected at the time of hypophysectomy into animals placed on a black background they will pale rapidly. However, the paling effect of the adrenalin soon wears off and the animal adapts completely to the black background. Subsequently pallor, due to disappearance of the dispersing factor of the pituitary, sets in and becomes maximum in 3 or 4 days as previously stated. The adrenalin test made several hours after hypophysectomy indicates that some dispersing substance from the pituitary is available to influence the melanophores about 70 hours after hypophysectomy.

C. Color Reactions of Hypophysectomized Catfishes to Darkness. When catfishes which had been hypophysectomized several days were placed with controls in darkness two interesting observations were recorded. (1) All the operated animals paled to a shade lighter than intermediate but somewhat darker than complete white-adaptation. Control fishes became intermediate in color. (2) Control animals were not all of the same shade but showed considerable variation about intermediate. The operated catfishes were remarkably homogeneous in their characteristic paler shade. The hypophysectomized catfishes when blinded still retain this pale shade in darkness.

D. Responses of Hypophysectomized, Blinded Catfishes to Illuminated

Backgrounds. When a normal catfish in an illuminated environment is blinded, it darkens rapidly to a coal black (Parker '34, Abramowitz '36). This reaction is pronounced (melanophore diameter 130 to 140 μ), and is not modified by background, but is reversed to complete pallor by darkness (absence of light). Adrenalin injection will also lighten an animal darkened by blinding.

It therefore seems significant that when a catfish, which has been hypophysectomized and kept on a *black* background 4 or 5 days, is blinded, it does not darken. However, if the animal has been kept on a *white* background for the same length of time since hypophysectomy, blinding results in slight darkening to a shade equal to a black-adapted hypophysectomized animal (melanophore diameter about 70 μ).

If blinding precedes hypophysectomy the expected results obtain. A fish made coal black by blinding will become pale following hypophysectomy. This pallor never exceeds the condition typical of black-adapted hypophysectomized fishes. Ways of lightening such an animal more completely are: (1) placing in total darkness (as noted under C), (2) by adrenalin injection or (3) electrical stimulation. Because the melanophore diameter (130 to 140 μ) of blinded catfishes is reduced to 70 μ by pituitary removal, it appears reasonable to conclude that the dispersing material of the pituitary is responsible for a major part in the darkening process in catfish color change.

If the coal black reaction in blinded animals is allowed to persist more than a few days before hypophysectomy, pigment may accumulate (Odiorne '37) in sufficient quantities to mask the pallor caused by hypophysectomy. However, pigment degeneration will finally take place in the hypophysectomized fish and the color behavior previously described will obtain.

E. Influence of the Injection of Catfish Pituitary on Color Behavior. The foregoing experiments were checked by injections of a suspension of finely minced catfish pituitaries into hypophysectomized fishes. The glands were taken from animals adapted to an intermediate background. It is hoped that the pituitaries of catfishes from various environmental conditions of background and illumination may be assayed for melanophore dispersing potency in future experiments. However, in this series of injections, dosages of from 1 to 4 pituitaries equivalent were administered subcutaneously into catfishes which had been hypophysectomized at least one week. The results of the injections may be summarized thus:

1. The melanophore diameters of black-adapted hypophysectomized fishes increased from about 70 μ to 125 μ.

2. Blinded, hypophysectomized catfishes under illumination darkened to coal black typical of illuminated, blinded animals. The melanophores enlarged somewhat more than noted in paragraph 1.

3. Hypophysectomized animals in darkness darkened markedly. This is probably not the normal physiology of the animal because the normal intact catfish pales in darkness to intermediate or lighter. This darkening is probably due to the sudden introduction into the circulation of abnormally large quantities of the dispersing substance which may be in such abundance that concentrating nerves are unable to over-balance its effect.

4. Any normal or hypophysectomized catfish in an illuminated environment may be darkened by pituitary injection as has been shown by Odiorne '33 and Parker '34. That the darkening will occur even if the animal is on a white background may be explained as in paragraph 3.

The effects of all the above injections are but temporary and vary in length with the dosage given. The largest injections lasted only about 4 days. An interesting aspect of injection experiments of this type is that experimental deficiencies may be cancelled out by substitution therapy.

Discussion.—Parker ('34) concluded that catfishes darkened by the combined action of dispersing nerves and a dispersing neurohumor from the pituitary which be believed to be weak in action. Abramowitz ('36) confirmed Parker's work and also stated that denervated cells do not readily attain a condition of maximum dispersion in black-adapted hypophysectomized animals.

That the dispersing substance of the catfish pituitary has hitherto been considered weak and insignificant in normal color change may be traced to the fact that the animals were not studied long enough after hypophysectomy (Parker's animals lived only 2 days). This same criticism has been voiced by Veil ('37) who believes that the rôle of the pituitary's dispersing agent is as significant in the catfish as it has been considered in the elasmobranchs by Lundstrom and Bard ('32).

It seems clear that the dark phase of the catfish is caused by more than one factor. The dispersing agent of the pituitary apparently causes about two-thirds of the darkening but the remaining third which occurs in the absence of the pituitary must be explained by other mechanisms. Parker ('34) believes that dispersing nerve fibers are the chief dispersing agents. Abramowitz ('36) and Wyckes (personal communication) are of the opinion that unstimulated melanophores assume a "resting" condition of partial dispersion.

Summary.—The experiments in this preliminary paper indicate that the pituitary of the catfish secretes a melanophore-dispersing agent which is the most important single darkening factor in its chromatic system. Tests show that functional amounts of the dispersing material remain in the circulation of the catfish until about 70 hours after hypophysectomy (at 12°C.). Hypophysectomy does not annihilate color change but limits the range of shades which the animal may assume. A normal catfish may white-adapt (with melanophore diameter 45 μ to 50 μ) or may black-adapt

(with melanophores dispersed to 125 μ to 130 μ). A blinded fish becomes coal black (melanophore diameter 130 μ to 140 μ) on any illuminated background. When the pituitary's dispersing substance is absent from the circulation, blinding or black background (strongest dispersing stimuli) are incapable of dispersing the melanophores to a diameter greater than 70 μ to 75 μ.

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ON THE DUPLEXITY THEORY OF VISUAL RESPONSE IN VERTEBRATES

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The conception that the visual performance of vertebrates in general involves the separable functioning of two morphologically distinct kinds of retinal sensory elements arose from the suggestion of Schultze (1866). Observations have tended on the whole to support the proposition that retinal "rods" are concerned with visual response at lower illuminations, "cones" with excitation at higher intensities of light,[1] as based upon correlations between retinal histology and the ethology of the animals concerned, their nocturnal or diurnal activities and other features of the illumination of their habitats during their active movements.[2] There are serious difficulties, however, in the way of the application of histological criteria for "rod" and "cone," even in a single retina, and of considering them as invariable types.[2] On the other hand the phenomena of their photomechanical and otherwise induced movements[3] and their "spontaneous" changes[4] frequently serve to separate the sensory cells of the vertebrate retina into two physiologically

distinguishable categories, corresponding to the histological types labelled "rods" and "cones," respectively.

Of a logically different order are the kinds of evidence obtainable from measurements of the visual performance of vertebrates. Among fishes[5] and amphibians[6] thus far examined, and in man,[7] having both rods and cones, the relation between flash-frequency (F) and critical illumination ($\log I$) for response to flicker is expressed as a double sigmoid curve (Fig. 1), a smaller one at lower intensities succeeded by a larger one at higher intensities. On the more or less general grounds already indicated, supported by the comparable evidence derived from the properties of diverse human visual functions,[8] these two portions of the composite graph, signifying the activities of two groups of excitation elements, have been labelled (for convenience and consistency in reference)[5,6,7] as respectively determined by the activities of rods and cones.

The identification would always remain defective, however, without some more direct demonstration. The occurrence of "double cones" in lower vertebrates is an example of the kind of complication which arises.[6] The functional proof of the existence of two chief populations of sensory elements does not necessitate that these are to be severally identified with "rods" and "cones." Nor can the comparison of the visual function contours provided by various animals with the numbers and proportions of the rods and cones apparent in their retinas really settle the answer in a satisfactory way. The evidence thus obtained, while certainly consistent with the requirements of the duplicity doctrine, does not establish its inevitability.

There is required for a sufficient test the quantitative description of the visual performance of vertebrates possessing only "cones," or only "rods," as structurally defined. It is true that the curves for visual functions of arthropods established in this way show no such separation into two parts as is indicated by the graphs in figure 1, and that one has no evidence of their involving two or more distinct sorts of receptor units;[9] but this evidence is not decisive. For another additional reason, it was important for our purpose to investigate the visual behavior of vertebrates exhibiting only one structural type of retinal element. The "composite" graphs of the vertebrate flicker contour have been separated into two portions[10] by fitting to the ascending parts two log-probability integrals. Reasons for assuming such a function, rather than one of the photostationary equilibrium type for example, are discussed elsewhere.[11] Such separations have involved extrapolation of the "cone" curve over some little distance toward the log I axis. If one were to make suitable measurements with a rod-free vertebrate, which seems thus far not to have been done, it should be possible to discover if (1) the absence of "rods" brings with it the absence of the lower section of the typically composite graph, and (2) if the complete curve is in

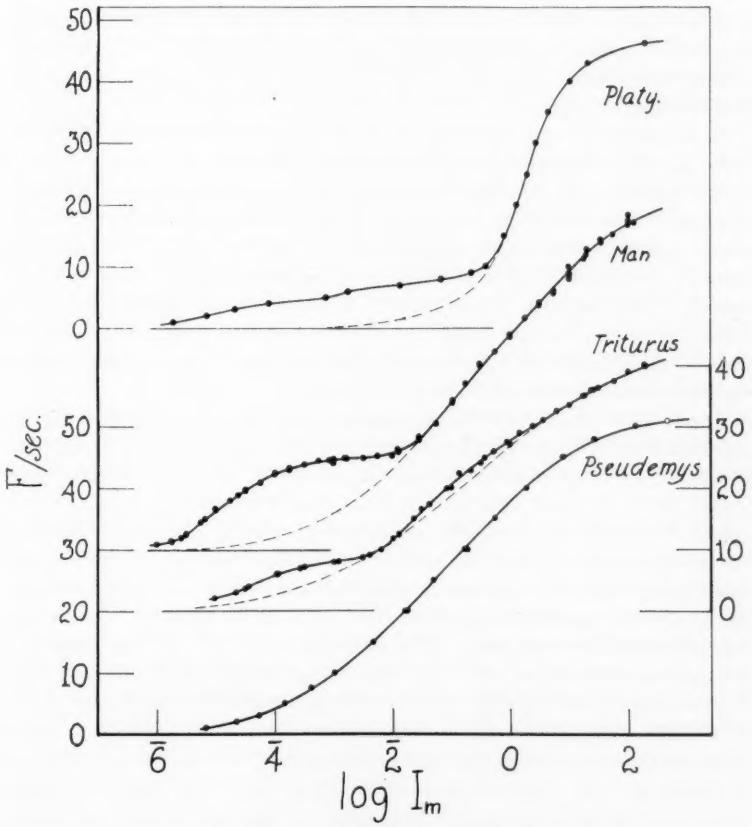

FIGURE 1

The relation between flash-frequency F and mean critical intensity for response to flicker, as obtained for several vertebrates, with a flash cycle in which the proportion of light-time to dark-time = 1. The curves for the fish *Platypoecilius*,[7a] the newt *Triturus*,[6] and the turtle *Pseudemys scripta* are based upon 30 observations at each point (3 on each of the same 10 individuals at each point, for each curve), at 21.5°; the curve for "man" gives averages of 10 observations for each point with one individual.[9] (The curve drawn for *Pseudemys* is a probability integral; the slight departures are due to the fact that the 10 individuals whose curves have been averaged do not form a truly homogeneous group; a better fit is obtained for the individuals taken separately. The "hump" on the *Triturus* curve is due to another kind of complication.[6] For the 3 upper curves the fitted probability integral has been extrapolated toward the log I axis; the difference between the observations and the extrapolated curve is attributed to the activation of rods, which beyond a certain intensity is no longer effective.)

fact adequately fitted by a log-probability integral over its whole extent. Experiments with a cone-free form would complete the formal argument, and provide certain tests of the "difference curves"[6,10] in the case of the flicker-response contour.

If the quantitatively separable portions of curves of visual functions are to be allocated respectively to the functional activities of rods and cones, a modification of the customary argument concerning the duplicity theory is required. The comparison of flicker-reaction contours in different vertebrates makes it clear that a separation into "rod" and "cone" categories is scarcely significant in terms of intensity thresholds alone. The primary question is whether the data indicate the presence of one homogeneous population of sensory effects, or of more than one such population. Visible structural properties of the peripheral elements may be of only slight meaning for this matter, even when unambiguous.

As in other cases of significant description of functional properties, the whole course of the property as a function of its inciting variable must be determined. Thus when the tentatively labelled "rod" contribution to the flicker curve of a particular animal is small, the spread of the "cone" portion is likely to be great and its slope low.[5,6,7] On the other hand, the converse relationship is not at all valid. It could not be said, in view of such facts, that a "rod"-poor retina is necessarily incapable of providing a receptor field for visual functioning at intensities so low as are possible with an animal well provided with rods. This curiously anti-"teleological" aspect of the general situation, which is emphasized by some additional observations, is related to the fact that the characteristic quantitative properties of "rod" and "cone" populations may be determined in separate and distinct ways by hereditary processes in a single type of animal.[5] Facts of the latter kind, of course, give an additional proof of the existence of two kinds of elements active in visual response, but do not prove that they are respectively due to "rods" and to "cones." The significant labels, which avoid possibly irrelevant reference to histological criteria, could be perhaps *photopic* and *scotopic* populations of sensory effects, when these are distinguishable; if only one such is to be detected in a given animal, it is to be characterized by its measurable parameters.

The majority at least of diurnal turtles, like certain other reptiles, are said to be remarkably free from rod visual cells.[12] We have utilized the reaction of young individuals of *Pseudemys scripta* to visual flicker, as observable in the apparatus we have employed for work with insects,[9] fishes,[5] amphibians[6] and man.[7] The details of the procedure are described elsewhere, together with certain further considerations arising from the data. Examination of sections of the retina of these animals shows the absence of identifiable rods. If such cells are present they are very few indeed.

The flicker-response curve in figure 1 was obtained from *Pseudemys*,

using a flash-cycle with equally long light and dark intervals, at 21°.5. It shows no trace of the composite character apparent in the usual flicker curve for a vertebrate. It is accurately described over its whole determinable extent by a single log-probability integral, which is the curve adjusted to the observations in figure 1.

The slope of the curve for *Pseudemys* is much lower than that obtained under the same conditions from various teleosts.[5] The intensity range covered is however as great, and the maximum to which it rises is of the general order encountered in all vertebrates. That in the absence of histologically identifiable retinal rods a smooth probability integral is obtained for the whole relation of log I to F is a fairly decisive argument for the duplicity theory and for its interpretation of the compound character of the corresponding curves obtained with other forms. It also justifies the procedure we have employed for the analytical separation of the cone- and rod-parts of the typically composite flicker curve[5,6,7] on the assumption that the contribution for which cone excitation is responsible should be, if separately visible over its lowermost portion, a complete probability integral in log I.

Summary.—The absence of rods from the retina of the turtle *Pseudemys scripta* is correlated with the absence of a "rod" portion in the curve for response to visual flicker. This gives a proof for the validity of the essence of the duplexity doctrine. It permits the logical correlation of the occurrence of rods in diverse retinas with the presence of a presumptive rod contribution to the measured contours of visual performance. For this turtle the *flicker frequency* vs. log *critical intensity* curve is a smooth probability integral over its whole determinable range; this justifies the method of analytical dissection which has been employed with the composite F-log I curves of other vertebrates.

[1] Cf. v. Kries, J., *Handb. norm. path. Physiol.*, **12,** 1, ii, 678–707 (1929).
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[2] Cf. Menner, E., *Zeits. vergl. Physiol.*, **8,** 761 (1928).
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[3] E.g., Laurens, H., and Detwiler, S. R., *Jour. Exp. Zoöl.*, **32,** 207 (1921).

[4] Welsh, J. H., and Osborn, C. M., *Jour. Comp. Neurol.*, **66,** 349 (1937).

[5] Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *J. Gen. Physiol.*, **20,** 211 (1936–37); *Ibid.*, **21,** 17 (1937–38).

[6] In course of publication: data on the Newt *Triturus*.

[7] Cf. Hecht, S., Shlaer, S., and Smith, E. L., *Cold Spr. Harb. Symp. Quant. Biol.*, **3,** 237 (1935).

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[8] Hecht, S., *Physiol. Rev.*, **17,** 239 (1937).

[9] Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Ibid.*, **20,** 363 (1936–37); *Ibid.*, **21,** 223 (1937–38).

[10] Crozier, W. J., *Proc. Nat. Acad. Sci.*, **23**, 71 (1937).
Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Ibid.*, 516 (1937).
[11] *Journ. Gen. Physiol.*, **20**, 393, 411 (1936–37); and subsequent reports on the effects of alterations of the flash cycle.
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ON THE LAW FOR MINIMAL DISCRIMINATION OF INTENSITIES. III

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1. The conception has been advanced that in properly designed measurements of a biological property the mean observed variation is due to the determinate variability of the performance of the organism,[1] rather than to experimental "error." This has a number of general consequences for the interpretation of such measurements, and is a significant factor in the choice of an explanatory mechanism. For example, response to visual flicker is a function of flash-frequency F and flash-intensity I; and it is found that

$$\sigma_F = k\sigma_I \cdot (dF/dI), \qquad (1)$$

where σ_F is the mean S. D. of the measurements of critical F at fixed values of I, σ_I that for the measured values of I at fixed levels of F.[2] Equation (1) may be derived on the assumption that the eventuation of the observed response of the reacting organism includes and evidences a variability which is primarily responsible for the measured σ_I and σ_F.

In general, a uniformly maintained end-point of reaction establishes the critical relationship between measurable variables A and B concerned in the evocation of response. The function connecting A and B exists in the form of a band defining the probability that repeated measurements of A as a function of B or the reverse, under similar conditions, will fall inside the band rather than outside it. An equation of the form of (1) then defines the nature of mean σ_A in terms of mean σ_B, and *vice versa*. The laws of the variation for A and for B will not commonly be of the same type.

2. It is important that the validity of equation (1) should be tested for a case in which the experimental manipulations are of the same kind when either A or B is the independent variable. This is most easily done with

measurements of visual intensity discrimination. With the discriminometer described elsewhere[3] a circular light image of intensity I (visual angle

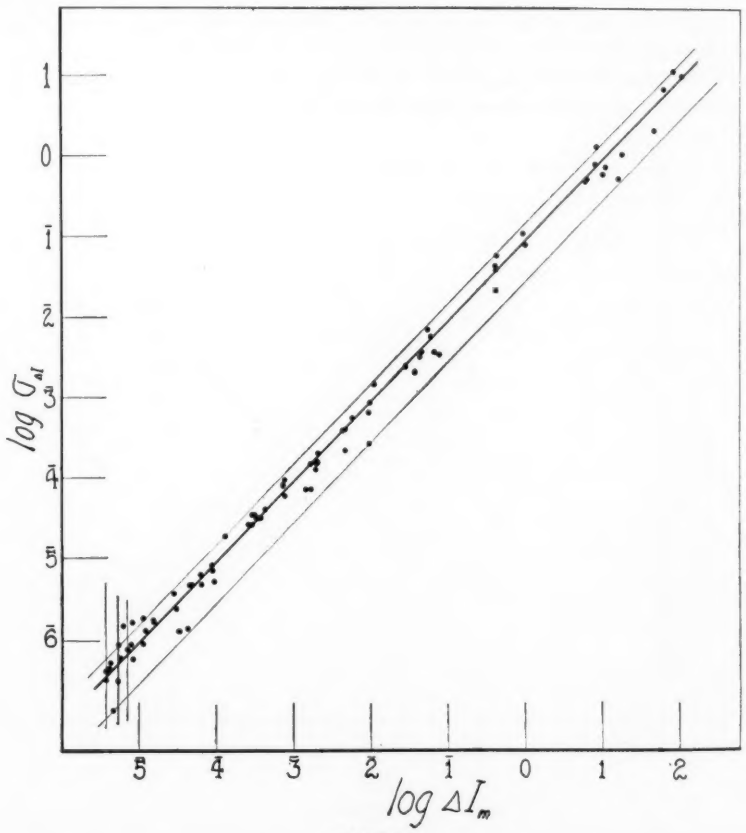

FIGURE 1

Data from measurements (one series) with $\Delta\bar{I}$ exposed as a flash (0.20 sec.); I_1 fixed and continuously exposed. Each point is a mean of 5 observations. The law is the same as for cases in which ΔI is obtained by continuous exposure: $\sigma_{\Delta I} = k\,\Delta I$. This series is homogeneous, and the central line halfway between the extremes of $\sigma_{\Delta I}$ divides the field into equal numbers of points. The mean position of the stimulus-threshold, with its scatter ($\Delta I \pm \sigma_{1\Delta I}$), is indicated by the vertical lines.

38°) is centered at the fovea on the retina. A second circular image (visual angle 16°) is added concentrically with the first. The larger area is then of intensity I_1, the smaller I_2 and $I_2 - I_1 = \Delta I$, where ΔI is in this case the

added intensity[1] required to evoke the end-point response. I_1 may be fixed, and ΔI can then be *increased* until difference between I_1 and $\bar{I}_2$ is detected. However in the reverse experiment the intensity of the 16° image can be fixed and I_1 then *lowered* until the difference between I_1 and I_2 is just detected. Since ΔI is added to I_1 to give I_2 we then have $\bar{I}_2 - \bar{I}_1 = \Delta I$. The instrumental manipulations producing adjustment of $\bar{I}_1$ and of $\Delta\bar{I}$, respectively, are of the same kind in the two cases.[3]

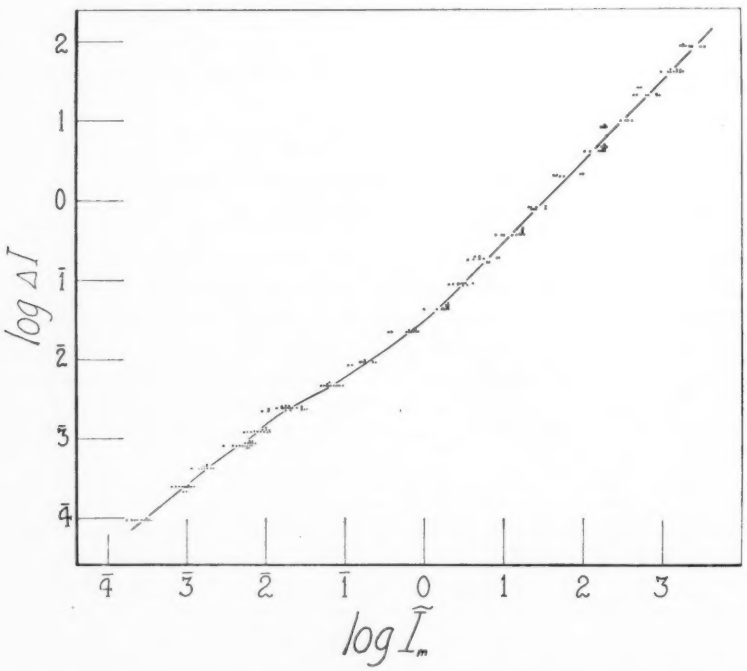

FIGURE 2

Log ΔI *vs.* log I. Solid dots, $(I_1)_m$ with $n = 5$, for fixed ΔI; the vertical span is constant, which means that $\sigma_{\Delta I}/\Delta I$ is invariant. At any level of ΔI the central line gives log $(\bar{I}_1)_m$.

The problem now is to predict the form of the variation of $\bar{I}_1$, as a function of mean $\bar{I}_1$ when ΔI is fixed, from knowledge about $\sigma\,\Delta I$ when I is fixed[4] on the assumption that the variation is due to the organism.

From (1) we have $\sigma_I = \sigma_{\Delta I}\,(dI/d\,\Delta I)$; dividing through by I,

$$\sigma_I/I = \sigma_{\Delta I}\,(d \log I\,/\,d\,\Delta I);$$

we know experimentally[5] that

$$(\sigma_{\Delta I})_m = k\,(\Delta I)_m,$$

hence

$$\sigma_I / I = K\,(d \log I / d \log \Delta I). \qquad (2)$$

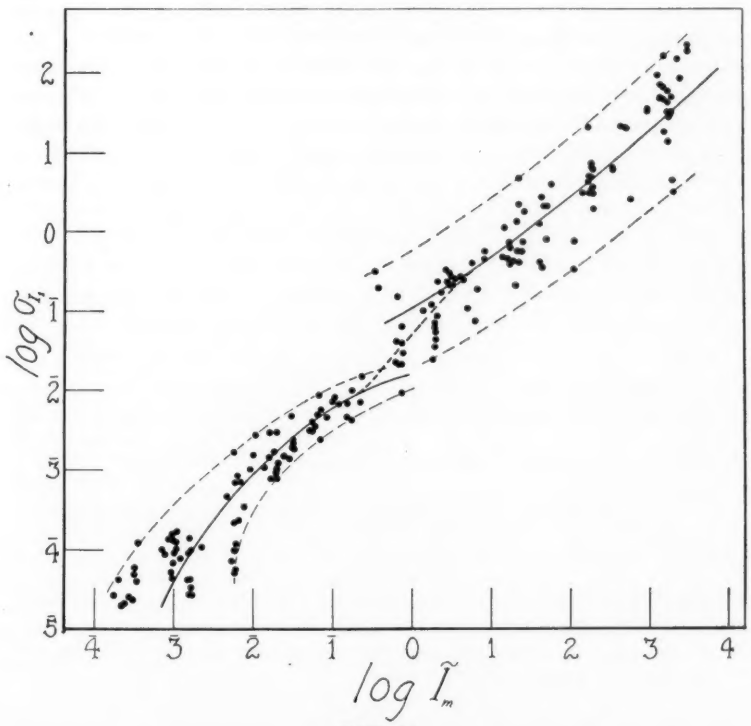

FIGURE 3

Log σ_I *vs.* log $\tilde{I}_1$. The graph is in two sections, the junction corresponding to the level at which discrimination becomes exclusively a "cone" function. The variation law is quite different from that for $\sigma_{\Delta I}$ (Fig. 1). The central line divides the plotted points equally; it does not give arithmetic division of the σ_I span (nor of the I_1 span), because five series of observations have been grouped.[6]

3. In the experiment devised to test this formulation the procedure was to present the adjusted intensity ($\tilde{I}_1$ or $\Delta\tilde{I}$) in a succession of 0.20 sec. flashes, with decrease ($\tilde{I}_1$) or increase ($\Delta\tilde{I}$) of intensity for the successive flashes until a difference between I_2 and I_1 was signaled as detected.[6] Sets of 5 such readings were averaged for each fixed value of ΔI or of I, and σ_1 computed for each set. Figure 1 shows that $(\sigma_{\Delta I})_m$ is a rectilinear func-

tion of ΔI_m, as in all such experiments.[5] Figure 2 gives the band form of log I_m as a function of log ΔI. The kink in the plot is attributable to the duplex constitution of the retina, the lower segment representing rod functioning, the higher that of cones;[7] the two are connected by a curved region, convex upward, suggesting rod-cone fluctuation of the sensory basis for the index-response in this region. (Individuals differ in the degree of this overlapping, and consequently in the curvature of the junction.)

The form of figure 2 requires that the variation of $\bar{I}$ must exhibit a course totally different from that of $\Delta\bar{I}$, if the variation observed is in each case due primarily to the lawful fluctuating capacity of the organism. The relative variation of ΔI $(=\sigma_{\Delta I}/\Delta I_m)$ is statistically constant (independent of I), and the vertical breadth of the band in figure 2 is therefore constant.

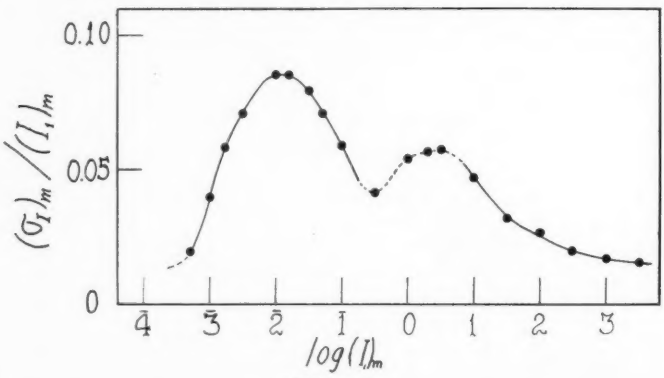

FIGURE 4

$(\sigma I)_m/(I)_m$ as a function of log $\bar{I}_1$, computed from the central line of figure 4. See text. To be contrasted with the properties of $\sigma_{\Delta I}$ (Fig. 1), where $(\sigma_{\Delta I})_m/(\Delta I)_m$ is found constant.

The horizontal breadth is not. We should find that as $\bar{I}$ increases σ_I/I_1 rises to a maximum, declines, then rises to a minor maximum, since the band of figure 2 exhibits an inflection on either side of a major curvature. From equation (2), σ_I/I must be inversely proportional at each point to the slope of the band in figure 2.

Figure 3 gives the relation between σ_I and $\bar{I}_m$. As a function of log I_m, log $(\sigma_I)_m$ rises, with declining slope, to a point corresponding to the exclusive dominance of presumed "cone" effects, then rises with increasing steepness. The vertical breadth of the band in figure 3 (a measure of σ_{σ_I}) is necessarily proportional at each point to the slope of the band, but the proportionality factor is not the same for the two parts of the curve and hence for the two "universes" of effects—traditionally associated with rods and

cones respectively—from which the samples of measurement are drawn.

4. The test of equation (2) is made by computing σ_I/I from the central line of figure 3. Five series of measurements are plotted together in figure 3. The assemblage is not exactly homogeneous;[1] because the whole function fluctuates from day to day, the central line does not vertically divide the σ_I span arithmetically in half.[5] Figure 4 shows that σ_I/I does exhibit two maxima, as predicted. If it were possible experimentally to determine the lowest part of the $\Delta I - I$ band more completely by the method of observing $\tilde{I}_1$ with ΔI fixed, which cannot be done because the visual threshold, the σ_I/I curve should rise again at the low I_1 end.

Summary.—When the minimal discriminable increment of intensity ΔI is measured as a function of $\tilde{I}_1$ (i.e., $I_2 - I_1 = \Delta\tilde{I}$), the relative variation of ΔI is constant (independent of I_1). If, using the same manipulative procedure, $\tilde{I}_1$ is determined as a function of fixed ΔI (by $\tilde{I}_2 - \tilde{I}_1 = \Delta I$), the relative variation of $\tilde{I}_1$ is directly proportional to $d \log I/d \log \Delta I$, and is a complex function of $\tilde{I}_1$. This result is uniquely predicted by the principle that the observed variation is due primarily to the fluctuating reactive capacity of the organism.

[1] Crozier, W. J., *Déterminisme et variabilité*, Paris, Hermann, 56 pp. (1935) (and citations to earlier work); *Jour. Gen. Physiol.*, **19**, 503 (1935–36); *Proc. Nat. Acad. Sci.*, **22**, 412 (1936).

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Holway, A. H., and Crozier, W. J., *Ibid.*, **23**, 509 (1937).

[2] Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **21**, 17 (1937–38).

[3] Crozier, W. J., and Holway, A. H. (in course of publication).

[4] It is to be noted[1] also that the curve of $\tilde{I}_m$ as a function of ΔI cannot be identical with that for ΔI_m as a function of I. In the present experiment the difference between the areas of I_1 and I_2 complicate the comparison.

[5] Crozier, W. J., *Jour. Gen. Physiol.*, **19**, 503 (1935–36).

Holway, A. H., *Jour. Opt. Soc. Amer.*, **27**, 120 (1937).

Crozier, W. J., and Holway, A. H., *Proc. Nat. Acad. Sci.*, **23**, 23 (1937).

[6] A full description of the procedure will be given elsewhere.

[7] Cf. Hecht, S., *Physiol. Rev.*, **17**, 239 (1937).

A DETERMINATION OF THE MAGNITUDE OF THE CELL "SENSITIVE VOLUME" ASSOCIATED WITH THE WHITE-EYE MUTATION IN X-RAYED DROSOPHILA. III

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Communicated February 8, 1938

In previous communications[1] we have discussed the procedures used and the results obtained in a probability determination of the magnitude of the volume and the radius of the cell "sensitive volume" which appears to be associated with mutation at the white locus in *Drosophila melanogaster*. It has been indicated that the result may be tentatively considered as the minimum limiting value for the size of the gene.

Partly as a test of the method, and partly because of the wealth of interesting results which it has given, we have recently extended the scope of the investigation to include (1) an evaluation of the mutation rate-dosage relationship as a function of temperature of the irradiated material, using a single wave-length of x-rays, (2) an evaluation of the same relationship at

TABLE Ia

(Males, 4°C.)

Exposure	Total number of eyes[a]	Number affected	Per cent affected	Probable error (percentage)	Calculated total sensitive volume
0 min.	4646	0	0.00	±0.000	
1/2 min.	2888	3	0.10	±0.040	14.66×10^{-18} cc.
1 min.	1769	4	0.23	±0.077	16.87×10^{-18} cc.
2 min.	8964	53	0.56	±0.055	20.53×10^{-18} cc.
4 min.	2010	20	1.00	±0.467	18.32×10^{-18} cc.

TABLE Ib

(Females, 4°C.)

Exposure	Total number of eyes[a]	Number affected	Per cent affected	Probable error (percentage)
0 min.	4746	2	0.042	±0.020
1/2 min.	2972	7	0.27	±0.064
1 min.	1870	10	0.53	±0.113
2 min.	9436	70	0.75	±0.060
4 min.	2035	29	1.41	±0.176

[a] It will be noticed that the total number of eyes given is sometimes odd. This situation results from the method used in counting. To facilitate rapidity, one eye only of each fly was counted in many cases. This, because of the random distribution of modified patches in right and left eyes, is considered a justifiable procedure.

constant temperature with variable x-ray wave-length, (3) a determination of the same function at constant wave-length and room temperature when the locus "garnet" is substituted for "white" and (4) for the "white" locus at room temperature under neutron bombardment of constant velocity distribution. The first experiment is considered sufficiently complete to justify its report at this time. Work is in progress on the remaining ones.

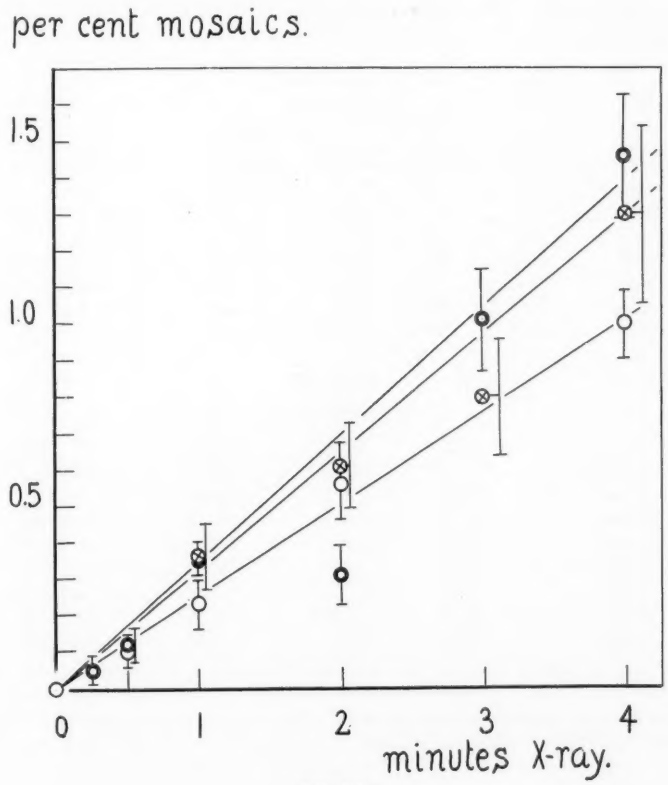


FIGURE 1

The experimental procedure reported in earlier papers was maintained unchanged, except that ova and young larvæ, eighteen to twenty-four hours old, were brought to temperature equilibrium with their surroundings at the values indicated before x-raying, held there during treatment and thereafter slowly returned to room temperature, at which the larvæ were reared. It was found possible to maintain eggs and larvæ at temperatures as low as

4°C. and as high as 54°C. Beyond these limits mortality resulting from temperature effects alone was unduly high.

The data of number of treated flies, number and percentage of affected eyes and percentage probable error involved, assembled separately for males and for females, are shown in tables I, II and III, for irradiation at temperatures of 4°C., 26°C. and 46°C., respectively. In figures 1 and 2 are shown the values for the percentage of affected eyes plotted arithmetically against the x-ray dosage. The justification of the arithmetic plot has earlier been considered.[1]

TABLE IIa

(MALES, 26°C.)

EXPOSURE	TOTAL NUMBER OF EYES[a]	NUMBER AFFECTED	PER CENT AFFECTED	PROBABLE ERROR (PERCENTAGE)	CALCULATED TOTAL SENSITIVE VOLUME
0 min.	4311	1	0.02	±0.016	
1/4 min.	1781	1	0.05	±0.036	14.66×10^{-18} cc.
1/2 min.	2583	3	0.11	±0.044	16.13×10^{-18} cc.
1 min.	4610	16	0.35	±0.054	25.66×10^{-18} cc.
2 min.	1960	6	0.31	±0.085	11.33×10^{-18} cc.
3 min.	2188	22	1.01	+0.144	24.68×10^{-18} cc.
4 min.	2237	32	1.46	±0.171	26.76×10^{-18} cc.

TABLE IIb

(FEMALES, 26°C.)

EXPOSURE	TOTAL NUMBER OF EYES[a]	NUMBER AFFECTED	PER CENT AFFECTED	PROBABLE ERROR (PERCENTAGE)
0 min.	4505	3	0.07	±0.026
1/4 min.	1716	2	0.12	±0.056
1/2 min.	2884	8	0.28	±0.066
1 min.	4616	23	0.50	±0.070
2 min.	2114	18	0.85	±0.135
3 min.	1740	26	1.49	±0.195
4 min.	2215	38	1.72	±0.186

[a] It will be noticed that the total number of eyes given is sometimes odd. This situation results from the method used in counting. To facilitate rapidity, one eye only of each fly was counted in many cases. This, because of the random distribution of modified patches in right and left eyes, is considered a justifiable procedure.

It will be seen that, as in the earlier experiments, a straight line may be considered the best fit for the points on all three of the curves representing data taken on males. This may be taken to indicate that the passage of a single electron through the locus considered is sufficient to accomplish the effect sought. As earlier set forth, this situation permits a calculation of the minimum limiting volumes of the sums of the loci for white eye in the x-chromosomes of all cells of the average optic anlage at the time of irradiation, at each of the three temperatures considered. These values, sepa-

rately calculated for each point on the three curves for males, are given in the final columns of tables Ia, IIa and IIIa.

The weighted means of these values, calculated in accordance with the

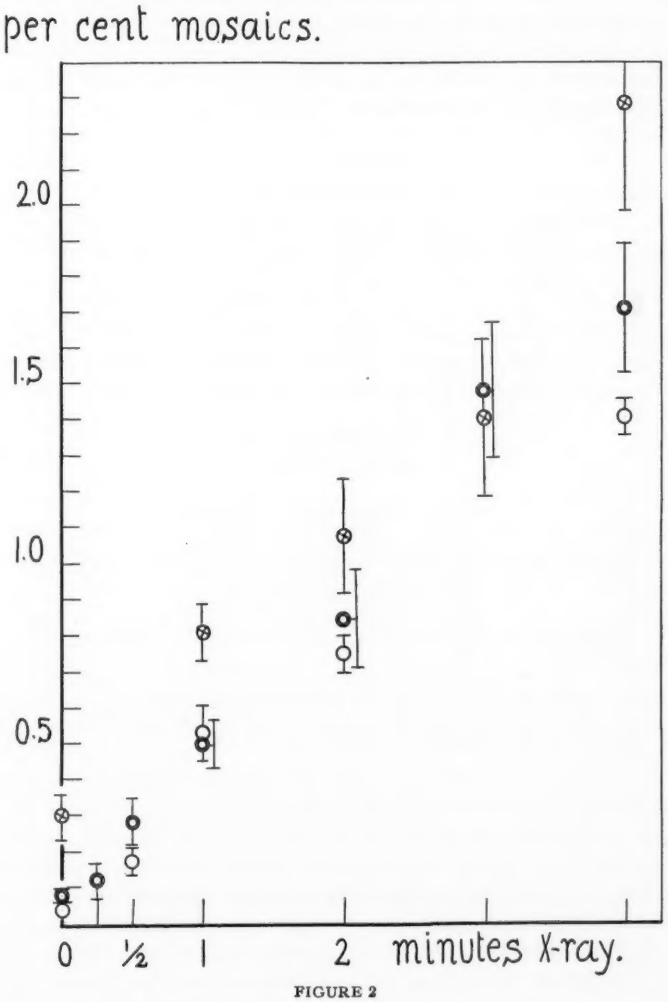


FIGURE 2

probable errors involved, are, respectively, 17.03×10^{-18} cc., 18.08×10^{-18} cc. and 23.36×10^{-18} cc. for 4°C., 26°C. and 46°C. Our earlier work indicated that the figure 15.6 most nearly represented the average number of

cells in the optic anlagen of eosin larvæ of the stock used at the time of irradiation. Dividing by this number yields 1.09×10^{-18} cc., 1.16×10^{-18} cc. and 1.50×10^{-18} cc. as values for the minimum limiting volumes of single loci for white eye at the three temperatures considered. The corresponding radii, assuming arbitrarily a spherical form for the locus, are, respectively, 6.39×10^{-7} cm., 6.52×10^{-7} cm. and 7.10×10^{-7} cm., somewhat less than our earlier determination of 7.62×10^{-7} cm. as the minimum limiting radius at room temperature.

TABLE IIIa

(MALES, 46°C.)

EXPOSURE	TOTAL NUMBER OF EYES[a]	NUMBER AFFECTED	PER CENT AFFECTED	PROBABLE ERROR (PERCENTAGE)	CALCULATED TOTAL SENSITIVE VOLUME
0 min.	2095	2	0.10	±0.045	
1 min.	1796	7	0.36	±0.095	26.39×10^{-18} cc.
2 min.	1812	11	0.61	±0.123	22.36×10^{-18} cc.
3 min.	1371	11	0.80	±0.162	19.55×10^{-18} cc.
4 min.	998	13	1.30	±0.241	23.80×10^{-18} cc.
6 min.	542	11	2.03	±0.408	24.80×10^{-18} cc.

TABLE IIIb

(FEMALES, 46°C.)

EXPOSURE	TOTAL NUMBER OF EYES[a]	NUMBER AFFECTED	PER CENT AFFECTED	PROBABLE ERROR (PERCENTAGE)
0 min.	1870	5	0.27	±0.079
1 min.	1840	15	0.81	±0.141
2 min.	1760	19	1.08	±0.165
3 min.	1216	17	1.42	±0.229
4 min.	1082	25	2.29	±0.307
6 min.	451	11	2.44	±0.480

[a] It will be noticed that the total number of eyes given is sometimes odd. This situation results from the method used in counting. To facilitate rapidity, one eye only of each fly was counted in many cases.

The deviations between the calculated radii at 4° and 26°, and 26° and 46° are, respectively, 1.3 and 5.8 Ångströms. These differences considerably exceed the maximum probable errors for the entire series of measurements. It is interesting and possibly suggestive that the value for the higher temperature is in each case the larger, and collateral experiments have been begun to check the effect in monomolecular protein films. However, we should prefer to regard the deviations as without significance at present, since we have never demonstrated that the probability method is capable of giving results reliable to more than an order of magnitude. It may yet be worth while to point out that the new values show a closer numerical agreement with the values of the "radii" of molecules of various pro-

teins obtained by ultracentrifuge methods, referred to in an earlier paper, than did the figure given at that time.

The form of the curves for the data from females is of some interest. It is, of course, far from rectilinear, and it has been tentatively considered that this may be due, at least in part, to a confusion of whitened patches resulting from gross chromosome aberrations in the anlage cells with those arising as a result of true "point-locus" changes. The end results of both effects might well be morphologically indistinguishable in females, while in the males nearly all of the former are automatically eliminated through the death of the affected cell, since it becomes heterozygous for a gross x-chromosome aberration. That this may be the correct interpretation is indicated by the relatively close correspondence of lower dosage points for the three temperatures, and the marked deviation in the higher ranges, possibly caused by the known high temperature coefficient of aberrations. Such an effect is notably absent in the plots of data from males. It has been evident from the first that data from females cannot be used in sensitive volume calculations, except in the case of especially balanced stocks.

Further work is in progress concerning this and related questions.

[1] Haskins, C. P., *Proc. Nat. Acad. Sci.*, **21**, 561–566 (1935).
Haskins, C. P., and Enzmann, E. V., *Ibid.*, **22**, 397–400 (1936).

THE SPECIFICITY OF PYRIMIDINE FOR PHYCOMYCES BLAKESLEEANUS

BY WILLIAM J. ROBBINS AND FREDERICK KAVANAGH

NEW YORK BOTANICAL GARDEN AND
DEPARTMENT OF BOTANY, UNIVERSITY OF MISSOURI

Communicated January 15, 1938

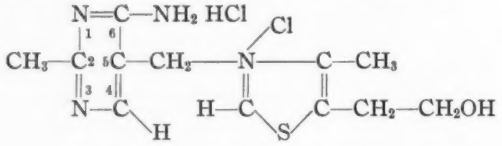
The authors[1] have reported that a mixture of the pyrimidene and thiazole intermediates used by Williams and Cline[2] in the synthesis of vitamin B_1 substitute for the vitamin in determining the growth of *Phycomyces Blakesleeanus* in a medium of mineral salts, dextrose and asparagine. Schopfer and Jung[3] and Sinclair[4] have found similar results. We have been interested in determining the specificity of the growth effect on *Phycomyces* of vitamin B_1 or its intermediates.

In an earlier paper[1] we reported negative results from the substitution for the vitamin thiazole of 15 sulfur compounds including some thiazoles. Negative results also were secured from the substitution of nucleic acid or its acid hydrolysate for the vitamin pyrimidine and in the substitution of pimelic acid or ethylene chlorhydrin for vitamin B_1.

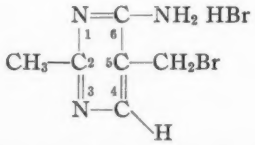
Since the previous report we have tested a number of additional compounds. Many of these compounds were secured through the generous cooperation of several individuals to whom we are deeply indebted.

The various pyrimidines tested were added to a medium ($MgSO_4 . 7H_2O$, 5 g.; KH_2PO_4, 15.0 g.; asparagine, 10.0 g.; dextrose, 100 g. and redistilled water, 1000 cc.) containing the vitamin thiazole. Twenty-five cc. of solution were used in 125-cc. Erlenmeyer flasks of Pyrex glass. Unless otherwise noted the solution and supplements were sterilized at 12 lbs. pressure for 20 minutes. After sterilization the solutions were inoculated with a suspension in redistilled water of the spores of *Phycomyces Blakesleeanus*, plus strain. For convenience in reference the various pyrimidines used or referred to are numbered.

The structure of vitamin B_1 hydrochloride is as follows:



On this basis 2-methyl-5-bromomethyl-6-aminopyrimidine hydrobromide is



We have used this system in naming the various pyrimidines referred to in this report.

We have reported previously that the 2-methyl-5-bromo-methyl-6-aminopyrimidine hydrobromide No. (1) and the 2-methyl-5-ethoxymethyl-6-aminopyrimidine No. (2) appear to be equally effective. A mixture of pyrimidine No. (1) or No. (2) and the vitamin thiazole was found[1] to be as effective in determining the growth of *Phycomyces* as molecularly equivalent quantities of vitamin B_1. Schopfer and Jung[3] and Sinclair[4] found similar results with 2-methyl-5-amino-methyl-6-aminopyrimidine No. (3).

We secured negative results with 2-methyl-5-ethoxymethyl-6-oxypyrimidine No. (4) and with 2-methyl-6-aminopyrimidine No. (5) supplied by Merck and Co., and with 2:6-dichloro-5-chloromethyl-4-methylpyrimidine No. (6) and 2:6-dihydroxy-5-hydroxymethyl-4-methylpyrimidine No. (7) kindly furnished by A. R. Todd of the Lister Institute of Preventive Medicine, London. Each of these compounds was used in amounts of 1 unit[5] and of 10 units with 10 units of the vitamin thiazole. A third compound

furnished by Todd, 2-methyl-5-thio-formamidomethyl-6-aminopyrimidine No. (8) was found partially to replace the vitamin pyrimidine. Sinclair found similar results but Schopfer[6] reports it to be ineffective.

The difference between the results of Sinclair and ourselves and those of Schopfer is probably because the effectiveness of this compound is influenced by heating. In our experiments it was most effective when filtered sterile, less effective if heated for 20 minutes at 12 lbs. pressure and ineffective after two periods of 20 minutes autoclaving at 12 lbs. pressure. Used with 10 units of the vitamin thiazole, pyrimidine No. (8) when filtered sterile yielded for 1 unit 49 mgms. dry weight of mycelium, for 10 units 189 mgms. and for 100 units 300 mgms. In this experiment the growth with 10 units of the vitamin thiazole and 10 units of pyrimidine No. (2) was 518 mgms. The loss of effectiveness of pyrimidine No. (8) on heating is evidence that the growth secured with it was not because of contamination by other pyrimidines. We have found pyrimidines No. (1) and No. (2) in acid or neutral solution to withstand several autoclavings, and Sinclair found pyrimidine No. (3) to withstand 2 hrs. at 125°C. even in the presence of 0.1 *N* NaOH. We estimate from our results the effectiveness of pyrimidine No. (8) to be 50% or less than that of pyrimidines No. (1), No. (2) or No.(3).

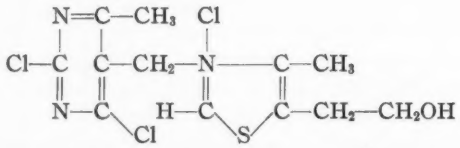
Ten units of each of the following pyrimidines were used with 10 units of the vitamin thiazole with negative results: 2:4 dimethyl-6-aminopyrimidine No. (9), 2:4 dichloro-5-chloromethyl-6 methyl pyrimidine No. (10), 2:4:6 trimethylpyrimidine dihydrate No. (11), 2-methylmercapto-4-methyl-6-hydroxypyrimidine No. (12), 2-phenyl-4-methylpyrimidine-6-carboxylic acid No. (13), 2-phenylpyrimidine 4:6 dicarboxylic acid No. (14), and 4-methyl-5 hydroxymethyl uracil No. (15). These seven compounds were furnished by Andrew Bowman of Oxford University.

A compound supplied by T. McLean of the Royal Technical College, Glasgow, 2-amino-5-methyl 1:3:4 thiadiazine hydrochloride No. (16), was used at 100 units with 10 units of the vitamin thiazole. Negative results were secured. The amount used was not entirely soluble. Negative results also were secured when compound No. (16) was used with 15 units of pyrimidine No. (1).

Fourteen pyrimidine compounds furnished by Treat B. Johnson of Yale University gave negative results. Ten units of each compound and 10 units of the vitamin thiazole were added to each flask. The compounds used were as follows: 2-thio orotic acid No. (17), 2-thio-4-methyl uracil No. (18), orotic acid No. (19), 5-iodo uracil No. (20), uracil-4-aldehyde No. (21), 2-thiouracil No. (22), 2-thio-4-methyl-uracil No. (23), uracil-5-carboxylic acid potassium salt No. (24), 1,4-dimethyl uracil No. (25), 2-thio-imino barbituric acid No. (26), 5-ethyl-4-methyluracil No. (27), 5-bromo uracil No. (28), 2-thio-4-hydroxymethyluracil No. (29), 4,5-dimethyluracil No. (30).

Schopfer[6] has reported negative results with the following additional pyrimidines: 2-methyl-6-mercaptopyrimidine No. (31), 2:5-dimethyl-6 aminopyrimidine No. (32), 2:5-dimethyl-6-oxypyrimidine No. (33), 2-methyl-4-amino-6-hydroxypyrimidine No. (34) and 2:6-dioxypyrimidine (uracil) No. (35). Sinclair's results with 2-methyl-5-thioformamidomethyl-6-hydroxypyrimidine No. (36) and the vitamin thiazole were negative.

A compound No. (37) resembling vitamin B_1 was supplied by Bowman. As shown by the structural formula below the thiazole portion of the compound is identical with the vitamin thiazole but the pyrimidine portion is not.



Used in amounts of 1 unit and 10 units this compound was ineffective. It was also ineffective when filtered sterile and used in an amount of 10 units. However, the growth with 10 units of compound No. (37) and 10 units of pyrimidine No. (2) was comparable with that obtained with 10 units of vitamin B_1. These results were secured also when the compound was filtered sterile. We conclude that *Phycomyces* probably splits the compound into thiazole and pyrimidine. The vitamin thiazole thus obtained is then combined with pyrimidine No. (2) to form vitamin B_1. Schopfer obtained similar results with two compounds which contain the vitamin thiazole; namely, 3-benzyl-4-methyl-5β-hydroxyethyl thiazole chloride and 3(4'[5']-methyimidazole)4-methyl-5β hydroxyethythiazole hydrochloride.

The following conclusions seem justified on the relation between the structure of pyrimidines and their effectiveness when used with the vitamin thiazole on the growth of *Phycomyces*.

1. The amino group in the sixth position is important. This follows from the negative results with pyrimidine No. (4) where the amino group is replaced by oxygen.

2. The mono-substituted methyl group in the fifth position is important. This follows from the negative results with pyrimidine No. (5) where H replaces the methyl group and with pyrimidine No. (32) where the CH_3 group replaces the mono-substituted methyl group.

3. Various radicals may be attached to the methyl group in the fifth position as shown by the equivalent results with pyrimidines No. (1), No. (2) and No. (3) where the groups in the fifth position are CH_2Br, $CH_2OC_2H_5$ and CH_2NH_2, respectively.

4. The effectiveness is limited to some extent by the radical attached to the methyl group in the fifth position as shown by the results with pyrimi-

dine No. (8) when the CH_2NHCSH group reduced the effectiveness of the pyrimidine 50% or more as compared with that of pyrimidnes No. (1), No. (2) or No. (3).

5. We have no evidence on the significance of the methyl group in the second position.

6. The pyrimidine portion of the vitamine B_1 molecule is highly specific for *Phycomyces*.

[1] William J. Robbins and Frederick Kavanagh, *Proc. Nat. Acad. Sci.*, **23**, 499–502 (1937).

[2] R. R. Williams and J. K Cline, *Jour. Am. Chem. Soc.*, **58**, 1504–1505 (1936).

[3] William H. Schopfer and Albert Jung, *Compt. Rend .Acad. Sci. Paris*, **204**, 1500–1502 (1937).

[4] H. M. Sinclair, *Nature*, **140**, 361 (1937).

[5] 1 unit is 10^{-9} Mole of the compound in question.

[6] W. H. Schopfer, *Bul. Soc. bot. suisse*, **47**, 460–464 (1937).

THE SPECIFICITY OF THIAZOLE FOR PHYCOMYCES BLAKESLEEANUS

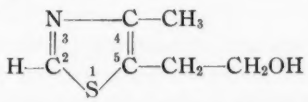
BY WILLIAM J. ROBBINS AND FREDERICK KAVANAGH

NEW YORK BOTANICAL GARDEN AND
DEPARTMENT OF BOTANY, UNIVERSITY OF MISSOURI

Communicated January 17, 1938

In a previous note[1] we have reported the results of a study of the specificity for *Phycomyces Blakesleeanus* of the pyrimidine portion of the vitamin B_1 molecule. In this paper the results with certain thiazoles are given.

The vitamin thiazole is 4-methyl-5β hydroxyethylthiazole No. (1) and may be represented as follows:



A mixture of the vitamin thiazole and 2-methyl-5-ethyoxymethyl-6-aminopyrimidine No. (1) or 2 methyl-5-bromomethyl-6-aminopyrimidine No. (2) is as effective as molecularly equivalent quantities of vitamin B_1 in determining growth of *Phycomyces*.[2]

Various thiazoles secured through the generous coöperation of various individuals were added to a medium ($MgSO_4 \cdot 7H_2O$, 5 g.; KH_2PO_4, 15.0 g.; asparagine, 10.0 g.; dextrose, 100 g. and redistilled water, 1000 cc.) containing pyrimidine No. (1) or No. (2). Twenty-five cc. of solution were used in 125-cc. Erlenmeyer flasks of Pyrex glass. Unless otherwise noted the solutions were sterilized at 12 lbs. pressure for 20 minutes. After steri-

lization the solutions were inoculated with a suspension in redistilled water of the spores of *Phycomyces Blakesleeanus*, plus strain. For convenience in reference the various thiazoles used or referred to are numbered.

Negative results were secured with the following thiazoles furnished by L. R. Cerecedo of Fordham University: 4-methylthiazole acetic acid No. (2), 4-methylthiazole-5-acetamide No. (3), ethyl-4-methylthiazole-5 acetate hydrobromide No. (4). Two amounts (1 unit[3] and 10 units) of each of these were used with 10 units of pyrimidine No. (1).

Four thiazoles furnished by H. T. Clarke of Columbia University were used. They were 4-methyl-5β-carbethoxy-ethylthiazole No. (5), 4-methyl-5β-ethoxyethylthiazole picrate No. (6), 4-methyl-5β-chloroethylthiazole picrate No. (7) and 2:4-dimethylthiazole pheniodide No. (8). Negative results were secured with thiazole No. (5) used in amounts of 1 unit and 10 units with 10 units of pyrimidine No. (1). Ten units of thiazole No. (8) with 10 units of pyrimidine No. (1) gave a yield of 3.3 mgms. dry matter. The results with thiazole No. (6) and No. (7) are shown in the following table. In each instance the thiazoles were used with 10 units of pyrimidine No. (1).

THIAZOLE NUMBER	AMOUNT UNITS	DRY WT. MYCELIUM MGMS. FILTERED STERILE	HEATED
No.(6)	1	0	0
	10	46	26
	100	669	650
No.(7)	1	0	8
	10	59	60
No.(1)			
Vitamin	1.0	...	80
Thiazole	10	...	484

Ten units of thiazolidine-4-carboxylic acid No. (9) prepared as described by Ratner and Clarke[4] were used with 10 units of pyrimidine No. (1) with negative results.

The following thiazoles were furnished by E. R. Buchman of the California Institute of Technology: 2:4-dimethyl-5β hydroxyethyl thiazole No. (10), 4-methyl-5β hydroxypropyl thiazole No. (11), and 4-methyl-5γ hydroxypropyl thiazole No. (12). Two amounts of each, 1 unit and 10 units were used with 10 units of pyrimidine No. (1). Thiazole No. (10) appeared somewhat toxic. Thiazole No. (11) was ineffectvie and thiazole No. (12) gave 2% or less of the growth of a molecularly equivalent quantity of the vitamin thiazole. Thiazoles No. (11) and No. (12) were filtered sterile and 100 units of each used with 15 units of pyrimidine No. (1). The growth was less than 1% of that secured with the vitamin thiazole.

T. McLean of the Royal Technical College, Glasgow, supplied 2 keto-4 methyl-2:3 dihydrothiazole-2-benzylidine hydrozone No. (13). Negative

results were secured when 100 units of this compound were used with 10 units of pyrimidine No. (2).

Schopfer[5] found the 4-methyl-thiazole No. (14), and the 4:5-dimethyl thiazole No. (15), were ineffective.

In addition we have tested nicotinic acid, neopeptone (Digestive Ferments Co.) and a sample of pantothenic acid kindly furnished by R. J. Williams. Nicotinic acid was found by Knight[6] to be a growth factor for *Staphylococcus aureus*. In our experiments both nicotinic acid and pantothenic acid gave negative results as a substitute for vitamin B_1, thiazole or pyrimidine. On the other hand some growth was secured when neopeptone was substituted for vitamin B_1 or its intermediates. As shown in the following table, there seemed to be more thiazole than pyrimidine in the sample of neopeptone used.

NEOPEPTONE	PYRIMIDINE NO. 1 UNITS	VITAMIN THIAZOLE UNITS	DRY WT. MYCELIUM MGMS.
0.1 g. per l.	0	0	11.3
0.1 g. per l.	10	0	30.5
0	10	10	350.0

The following conclusions seem justified on the relation between the structure of thiazole and its effectiveness when used with a suitable pyrimidine on the growth of *Phycomyces*.

1. The H in the second position is important. This follows from the negative results with thiazole No. (10) in which the H is replaced by CH_3.

2. The β hydroxyethyl group in the fifth position is important. This follows from the negative results with thiazole No. (2), No. (3), No. (4), No. (5), No. (11), No. (12), No. (14) and No. (15) in which the β hydroxyethyl group is replaced by the acetate, acetamide, ethylacetate, β carbethoxyethyl, β hydroxypropyl, γ hydroxypropyl, hydrogen or methyl radicals respectively.

3. The hydroxyl in the hydroxyethyl group is important. This follows from the materially decreased growth with thiazoles No. (6) and No. (7) in which the hydroxyl is replaced by the ethoxy radical or by chlorine.

4. We have no evidence as yet on the significance of the CH_3 group in the fourth position.

5. The thiazole portion of the vitamin B_1 molecule is highly specific for *Phycomyces*.

[1] William J. Robbins and Frederick Kavanagh, *Proc. Nat. Acad. Sci.*, **24**, 141–145 (1938).

[2] William J. Robbins and Frederick Kavanagh, *Ibid.*, **23**, 499–502 (1937).

[3] 1 unit is 10^{-9} Mole of the compound in question.

[4] Sarah Ratner and H. T. Clarke, *Jour. Am. Chem. Soc.*, **59**, 200–206 (1937).

[5] W. H. Schopfer, *Bul. Soc. bot. suisse*, **47**, 460–464 (1937).

[6] B. C. J. G. Knight, *Biochem. Jour.*, **31**, 966–973 (1937).

NOTE ON THE PROBLEM OF THE EXPANDING UNIVERSE

BY HARLOW SHAPLEY

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Communicated February 14, 1938

1. Question as to the proper interpretation of the red-shift has been raised through the publication of nebular counts by Hubble.[1] If we assume that the galaxies are in the main uniformly distributed in space, we have the relation $\log N_m = 0.6\ (m - m_1)$ between the number of objects per square degree and the apparent magnitude limit m (corrected for red-shift). The constant m_1 is the space-density parameter, that is, the magnitude to which a survey must penetrate to record on the average one object per square degree. Its evaluation is necessary in the calculation of the effect of red-shift on magnitude; and the size of this effect determines whether or not the red-shift means an expanding universe, or demands some other as yet unspecified interpretation.

The value of m_1 cannot be very securely determined by means of the thirteen hundred galaxies brighter than the thirteenth magnitude, because not only is the number involved relatively small, but also the distribution is dominated in some parts of the sky by clusters of galaxies, and in others by irregular space absorption. Nor can m_1 be precisely determined as yet by the use of objects fainter than the nineteenth magnitude, where difficulties arise through the increasing size and significance of the red-shift correction and increasing uncertainty in the nebular magnitude scale.

Between the thirteenth and nineteenth magnitudes the red-shift correction is either negligible or not seriously uncertain, and in that interval the stellar photographic magnitude scale, on which the nebular magnitudes are based, is most satisfactorily determined. It is here, therefore, that m_1 can best be derived from nebular counts and from photometric measures.

A recently completed survey of the galaxies to the eighteenth magnitude in the south galactic polar cap provides extensive material for the determination of m_1. Also this new survey gives a picture of the distribution of galaxies over an extended region of the sky that appears to be quite clear of irregularities in space absorption and not obviously affected by distinct clusters of galaxies. The details of the investigation will be presented in Circulars and Annals of the Harvard Observatory; the present account will deal only with the determination of m_1 and related quantities.

2. Eighty plates of three hours exposure have been made in the polar area bounded by galactic latitude $-55°$; all but nine are in the zone bounded by $\beta = -60°$. The plates are of fairly uniform quality, the mean limiting stellar magnitude being $m_s = 18.36 \pm 0.04$ (m.e.). Each plate, fourteen inches by seventeen inches, covers approximately thirty-five

square degrees. In the central part of the plate (three degrees on a side) there is no appreciable magnitude loss that depends on distance from the optical axis, for either stars or galaxies. In the following discussion of the nebular counts and the nebular distribution, the argument is based chiefly on these central nine square degrees, in which we find on the average about fifty galaxies per square degree.

In the surrounding sixteen square degrees we find, in the mean, only thirty-one galaxies per square degree, indicating that the limiting magnitude for galaxies at this distance from the center of the plate is between three- and four-tenths of a magnitude brighter than at the plate center. These outer squares can be used effectively in studying the unevenness of the nebular distribution from plate to plate over the polar zone, as well as in the general cataloguing of external galaxies.

3. The plates for the present survey were made with the 24-inch Bruce doublet at Bloemfontein. The magnitudes have been determined on each plate separately by the star-count method. The nebular counts are summarized in table 1.[2] The last column gives for each plate the number of galaxies per square degree averaged over the central nine square degrees after the plate is reduced to a common magnitude limit. This reduction has not appreciably affected the total numbers involved, and has decreased the differences in magnitude limit from plate to plate that arise from variations in sky conditions and emulsion speeds. Since the magnitudes are determined independently on each plate, no adjustment is needed for differences in zenith distance.

4. From the 36,274 objects actually observed within the 9-square areas of the eighty plates, we deduce, when each plate is treated as a uniform unit, the mean value and its mean error

$$\overline{N}_m = 51.51 \pm 3.11$$

The limiting nebular magnitude m is found (from a study of the magnitude-frequency plots for the galaxies on a hundred long-exposure Bruce plates, mostly in the zone[3] south of declination $-60°$) to be 18.20 with an estimated mean error of less than $0^m.05$. The red-shift correction is taken as $0^m.16$ (from a discussion by Hubble), and the corresponding space-density parameter is computed to be

$$m_1 = 15.19,$$

with an estimated mean error of $\pm 0^m.06$. This value of m_1 is in fair agreement with the values of 15.3 and 15.09 derived by Mayall[4] and Hubble,[5] respectively. All three determinations, of course, are computed on the hypothesis of uniform space density.

The standard deviation from the mean $\overline{N}_m$ for a single plate in table 1 is 27.67, indicating the considerable irregularity in nebular distribution

throughout the south galactic cap, even when areas as large as nine square degrees are involved. The distribution of $\overline{N}_m$ is of course not symmetrical, the quartile, median and three-quartile values being 30.2, 44.4 and 64.0, respectively. The mean of the three largest values of $\overline{N}_m$ is ten times the mean of the smallest three, corresponding, on the uniform space-density hypothesis, to a difference in m_1 of 1.67 magnitudes—a difference that is quite out of the question as an error in determining magnitude limits, and indicates rather the degree of non-uniformity that may be found when neighboring areas in high latitudes, each of twenty-seven square degrees, are compared.

5. Because of the clustering tendency of galaxies, it is best not to examine the spread of values of the parameter m_1 only on the basis of richest and poorest plates, but rather to deal with large areas. If we divide the polar cap into eastern and western halves, containing respectively the second and third longitude quadrants and the first and fourth longitude quadrants, we derive from the data of table 1 the results of table 2. Again each plate is treated as a uniform unit and the data are used from the central nine square degrees only.

TABLE 2

	EAST	WEST
Number of plates	38	42
Area in square degrees	342	378
Total number of identified galaxies	19,617	16,657
Mean $\overline{N}_m$ (reduced to $18^m.2$)	60.82	43.14
Standard deviation	33.47	25.90
Mean error of mean	5.50	4.04
Median $\overline{N}_m$	51.75	37.15
Quartile values of $\overline{N}_m$	39.4, 69.6	26.7, 51.8
Mean of 3 largest $\overline{N}_m$	154.5	104.9
Mean of 3 smallest $\overline{N}_m$	20.2	15.7
Space-density parameter m_1	15.07	15.32

A similar difference in the values of m_1 would be obtained if we divided the polar area into northern and southern halves; but it is better, in order to avoid a possible systematic error, to divide as above, because the stellar magnitude limits have been deduced by slightly different methods for regions north and south of declination $-23°$. There appear to be no factors of an observational, seasonal or computational nature that can account for an appreciable amount of the differences in the nebular count for the two half-zones. We conclude that the observed difference in average population per square degree, namely 60.82 − 43.14, is an indication of a metagalactic structural condition. The eastern half of the absorption-free polar cap is 1.41 times as rich as the western half.

6. It should be noted that the areas covered, 342 and 378 square degrees, as well as the numbers of galaxies observed, 19,617 and 16,657, are

considerably larger than the areas and numbers in any of the five surveys made with the reflectors at the Mount Wilson and Lick Observatories that have been used by Hubble in the analyses of the distribution of galaxies in space. From Hubble's table 1 in the paper referred to above[1] we derive the following values:

To magnitude limit	18.47	19.0	19.4	20.0	21.03
Total of nebulae actually identified	2,299	9,940	8,774	11,856	8,200
Total effective area* in square degrees	30	(69)	40	24.3	5.6

* The "effective" area for a survey, in square degrees, is the average number of nebulae actually identified per plate, divided by the average number per square degree (reduced to standard conditions) multiplied by the number of plates.

Except perhaps for the two brightest limits, the intercomparison of areas involved is not significant. It is the volumes of space that should be intercompared, or, better yet, the total number of galaxies observed.

7. When the four quadrants of the south galactic cap are treated separately, we find from the Harvard material:

Quadrant	1	2	3	4
Number of plates	18	18	21	23
Number of galaxies	5,002	8,708	10,909	11,655
m_1	15.54	15.15	15.00	15.19

8. The material discussed above from the Harvard survey can be more than doubled by using the 39,680 other galaxies that are observed on the same plates in the sixteen square degrees outside the central nine square degrees. When the plates are reduced as before to a common stellar magnitude limit, the average number of galaxies in this outer area per square degree (each plate again treated as a uniform unit in computing the uncertainty) is

$$\overline{N}_m = 33.03 \pm 2.18 \text{ (m.e.)}.$$

The standard deviation for one plate is 19.36. The mean nebular magnitude limit for the sixteen square degrees is approximately 17.87 instead of the 18.20 applicable to the central nine square degrees. This value is arrived at simply by the comparison of the nebular counts:

$$m_9 - m_{16} = 1.67 \log (\overline{N}_9/\overline{N}_{16})$$
$$m_{16} = 18.20 - 1.67 \log (51.5/33.0)$$

Since uniform space density is assumed for this calculation of m_{16}, the values of $\overline{N}_m$ and m_{16} have no independence in the computation of the parameter m_1; but the data from these outer sixteen square degrees for the eastern and western halves of the polar cap can be used with high weight for an independent examination of the values of m_1 obtainable in two large adjoining areas. Table 3 contains the material for the outer

areas, comparable in detail with table 2. The eastern half is 1.50 times as rich as the western half, and the corresponding difference in the space-density parameter is $0^m.30$.

TABLE 3

	EAST	WEST
Number of plates	38	42
Area in square degrees	608	672
Total number of identified galaxies	22,810	16,870
Mean $\overline{N}_m$ (reduced to $17^m.87$)	40.07	26.67
Standard deviation	22.53	13.01
Mean error of mean	3.70	2.03
Median $\overline{N}_m$	37.9	24.0
Quartile values of $\overline{N}_m$	25.0, 43.1	16.9, 30.7
Mean of 3 largest $\overline{N}_m$	100.9	56.7
Mean of 3 smallest $\overline{N}_m$	11.9	9.1
Space-density parameter m_1	15.06	15.35

9. On the hypothesis of strictly uniform space density, m_1 is a constant and the observed differences in $\overline{N}_m$ would need to be attributed to corresponding differences in the magnitude limit m. But discrepancies of this size and systematic character in the magnitude limit for adjacent parts of the polar cap are not credible. The fault undoubtedly lies in the assumption of uniform space density. The observed inequality can be found also in Hubble's survey to magnitude 18.47, since twenty of his reflector plates are in the first, twenty-two in the second quadrant, with latitudes between $-55°$ and $-90°$. He records 352 objects in the first, 562 in the second, and we find that the ratio of East to West is 1.45 for equal areas, in agreement with the results from Harvard plates (Sections 6 and 8 above). It may be noted that still larger inequalities are known than those in the south galactic cap. We hope to make a report soon on one or two of the great metagalactic clouds.

10. Finally we consider the bearing of the new data on the question of the effect of the red-shift on magnitude, given by the relation $\Delta m = B\frac{d\lambda}{\lambda}$. The factor B should be approximately 4.0 if the red-shift is due to recession, and 3.0 if recession is not involved. Both Eddington[6] and Hubble[7] have pointed out that B could be changed from Hubble's derived value of 2.94 to the value 4.0 with a relatively small change in the interval δm between the assigned magnitude limits of the various surveys. Thus Hubble notes that "an error in δm of the order of $0^m.12$, evenly divided between the two extreme limits, is required to furnish $B = 4.0$."

For his calculations at the brighter limit, 18.47, Hubble determines log N_m from the counts of 2300 objects. When the new survey at a corresponding bright limit is used for deriving m_1 and B, we find the 42,400 galaxies of the eastern half of the south galactic cap give a value of m_1 that is nearly

three-tenths of a magnitude different from the value derived for the 33,500 galaxies of the western half. The same difference carries over to m and δm. It appears, therefore, that the coefficient B cannot be accurately determined from existing data, and that we have no need as yet, from nebular

TABLE 1

CENSUS OF 75,900 GALAXIES IN THE SOUTH GALACTIC POLAR CAP

GALACTIC LONGITUDE	GALACTIC LATITUDE	N_{tot}	N_9	N_{16}	$\overline{N}_9$	GALACTIC LONGITUDE	GALACTIC LATITUDE	N_{tot}	N_9	N_{16}	$\overline{N}_9$
1.5	−67.3	971	363	456	53.2	207.0	−57.3	581	193	288	85.3
2.7	−60.3	486	166	231	36.9	207.1	−78.2	382	157	144	15.2
7.8	−76.2	250	142	99	27.4	213.4	−75.5	1054	431	480	47.9
14.2	−72.8	262	104	99	46.0	214.2	−62.6	1538	665	668	64.3
14.4	−80.6	666	235	324	30.0	222.4	−69.9	310	125	131	27.8
23.3	−63.4	802	300	381	25.3	226.3	−57.4	658	260	290	66.2
24.2	−77.8	255	101	104	16.9	226.5	−75.7	1046	448	485	49.8
39.4	−64.0	385	212	145	17.9	227.7	−63.8	1917	930	800	51.7
39.6	−71.1	1344	537	641	45.3	235.0	−55.6	1226	503	523	147.0
51.6	−56.2	378	131	190	25.3	238.8	−75.2	1463	650	628	72.2
55.1	−63.1	530	214	253	23.8	239.0	−72.1	798	420	331	46.7
60.5	−69.7	645	278	288	23.5	239.7	−69.2	1830	767	808	64.7
64.4	−83.9	739	284	316	20.8	240.3	−81.8	1772	668	840	18.4
68.0	−76.4	1877	793	847	44.0	252.4	−60.7	2208	784	1097	151.6
68.7	−60.6	684	274	335	15.2	253.7	−66.5	1818	658	878	63.6
76.9	−66.8	855	270	427	39.6	258.6	−72.5	1632	561	876	31.2
88.5	−72.3	687	293	279	37.4	264.3	−62.5	1197	514	533	99.5
89.5	−62.3	896	308	356	45.2	270.3	−67.7	1968	832	896	92.4
90.0	−87.8	2631	863	1489	63.3	270.9	−77.7	1931	807	830	68.1
102.8	−66.6	583	216	294	55.0	274.1	−55.0	896	328	432	31.7
110.1	−76.6	4519	1730	2127	48.1	274.8	−87.7	1730	807	712	34.1
110.5	−60.4	1318	466	640	51.8	276.3	−62.4	1954	812	838	90.2
111.9	−63.1	949	415	419	69.6	282.1	−72.1	864	335	340	42.8
120.3	−70.2	962	317	479	40.5	286.9	−66.4	2575	1102	1106	80.8
124.7	−63.0	905	399	416	38.6	287.7	−60.6	1026	451	372	66.1
127.3	−56.5	811	289	412	32.1	299.3	−63.0	4025	2050	589	132.1
139.6	−71.4	1040	409	464	60.0	300.8	−68.9	419	169	208	16.3
140.5	−64.2	794	264	413	38.7	302.1	−75.0	1457	617	644	39.8
141.5	−82.1	678	227	339	57.8	315.8	−60.9	1087	465	484	89.9
147.2	−85.0	968	355	457	30.0	316.7	−78.7	1043	422	474	54.0
149.9	−55.5	717	280	350	27.1	318.2	−69.2	981	328	450	36.4
156.1	−63.7	2010	853	897	164.9	325.5	−62.3	785	316	337	26.7
156.8	−78.1	457	228	184	50.7	326.6	−75.1	603	226	268	43.7
160.8	−71.1	664	230	345	51.2	331.6	−71.3	890	359	433	30.3
170.6	−62.1	1927	1012	689	56.2	334.8	−67.5	696	245	333	35.9
178.5	−67.8	479	155	262	39.4	337.6	−55.1	946	309	483	51.8
192.0	−72.6	1215	527	564	77.3	342.1	−61.7	203	61	114	15.5
192.1	−63.8	1725	861	730	83.2	342.5	−80.7	547	205	242	30.1
197.2	−81.0	271	73	128	42.6	347.7	−72.3	666	264	279	38.7
202.6	−59.5	1824	714	912	91.2	352.4	−60.6	486	145	235	28.0

counts, to question the interpretation which attributes red-shift to recession.

The result can best be shown numerically, through least-squares solutions for m_1 and B, which are related (when we adopt the velocity-distance factor derived at Mount Wilson) by the formula[8]

$$m_1 = m_0 - 1.667 \log \overline{N}_m - 10^{0.2\,(m_0 - \Delta m_0)} + \log B - 4.707$$

where m_0 and Δm_0 are the observed magnitude limit and the red-shift effect. We use all of the survey material presented by Hubble (Section 6 above) except that we substitute the Harvard counts to $18^m.2$ (nine inner squares only) for his data at the brighter limit, and obtain the following results for the eastern and western parts of the galactic cap:

	EAST	WEST
$\log \overline{N}_m$	1.784	1.635
m_1	15.01	15.26
B	3.48	1.81

If we should assume that the limit of completeness of the Harvard survey is 18.1 instead of 18.2, we find:

	EAST	WEST
m_1	14.90	15.19
B	4.2	2.2

[1] See, for example, these PROCEEDINGS, **22,** 621–627 (1936).
[2] Detailed tabulations are given in Harvard Circular 423 (*in press*).
[3] *Harv. Ann.*, **105,** No. 8, p. 137 (1937).
[4] *Lick Obs. Bull.*, **16,** 177 (1934).
[5] *Mount Wilson Contr.* No. 557, p. 15 (1936). Hubble's C is $-0.6\ m_1$.
[6] *Mon. Not. R. A. S.*, **97,** 156 (1937).
[7] *Ibid.*, **97,** 506 (1937).
[8] The form of the exponential term is suggested by Hubble.

DEPENDENT PROBABILITIES AND SPACES (L)

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1. *Introduction.*—The purpose of the present paper is to express the theory of dependent probabilities in new terms.

These terms are: (*a*) the general theory of linear operators on Banach space, and (*b*) the relation $f < g$. The abstract theory of partially ordered Banach spaces has already been formulated by Kantorovitch;[1] it involves such notions as: upper bound, lower bound, least upper bound or sup,

greatest lower bound or inf, lim sup, lim inf, positive part, negative part, absolute value and disjointness.

Firstly, the fundamental definitions are stated in a form which includes all known cases; this has never been done before. Secondly, Markoff's fundamental theorem on "probabilities in chain" is proved in a form including all known cases. And lastly, a new theorem, which specializes in the *deterministic* case to von Neumann's well-known Mean Ergodic Theorem,[2] is proved with added generality in the *stochastic case.*

The present paper merely sketches the proofs, which will be given in full elsewhere.

2. *Postulates.*—The model with which we shall work is described in the following:

DEFINITION 1: *By a space* (L), *we mean any space* Σ *which satisfies the following six postulates.*

P0: Σ is a linear space with real scalars, and a relation $f > 0$ (to be read, f is positive), is defined on Σ.

P1: If $f > 0$ and $g > 0$, then $f + g > 0$.

P2: If $f > 0$ and λ is a scalar, then $\lambda > 0$ implies $\lambda f > 0$ and conversely.

P3: Relative to the definition, $f > g$ (read, f is greater than g) means $f - g > 0$, Σ is a *lattice.*[3]

P4: A "norm" $\eta(f)$ is defined on Σ, relative to which Σ is a Banach space.[4]

P5: Norm is additive on positive elements; $f > 0$ and $g > 0$ imply $\eta(f+g) = \eta(f) + \eta(g)$.

Example: Let B denote the Boolean algebra of all subsets X of a class I_n of n points, or of Borel subsets of an interval I (or of any region!) modulo sets of measure zero. Then the additive, continuous functions defined on B satisfy (P0)–(P5), if by $f > 0$ we mean $f(X) \geqq 0$ for all X and $f(I) > 0$, and by $\eta(f)$ we mean $f(I)$ when $f > 0$ and sup $|f(X)| + |f(X')|$ in general Thus the space (L) and its finite-dimensional analogues are "spaces (L)."

Consequences of (P0)–(P2): Define $f > g$ to mean $f - g > 0$. Then (1) Σ is a partially ordered set in the sense of Hausdorff, (2) translations $x \to x + a$ preserve order, (3) homothetic expansions $x \to \lambda x$ preserve or invert order according as $\lambda > 0$ or $\lambda < 0$. We note that by (1)–(2), (P3) follows from (P0)–(P2) and the assumption that any element f has a "positive part" $f^+ = f \cup 0$, such that $x \geqq f^+$ implies $x \geqq 0$ and $x \geqq f$ and conversely.

Consequences of (P0)–(P3): If we set $f^+ = f \cup 0$, $f^- = f \cap 0$, and $|f| = f^+ - f^-$, then (4) the "Jordan decomposition" $f = f^+ + f^-$ holds, whence $f + g = (f \cup g) + (f \cap g)$ by (2), (5) $f^+ \cap (-f^-) = 0$—in words, f^+ and $-f^-$ are *disjoint,* (6) the dual distributive laws $f \cup (g \cap h) = (f \cup g) \cap (f \cup h)$ and $f \cap (g \cup h) = (f \cap g) \cup (f \cap h)$ are valid, (7) the triangle law on absolute values holds: $|f - g| + |g - h| \geqq |f - h|$, (8) the functions $f \cup g$ and $f \cap g$ are *monotone* in both variables, and *uniformly continuous* in that $|(f \cup g) - (f^* \cup g)| \leqq |f - f^*|$ and $|(f \cap g) - (f^* \cap g)| \leqq |f - f^*|$.

Consequences of (P0)–(P5): (9) The functions $f \smile g$ and $f \frown g$ are metrically continuous, in virtue of (8), (10) the functional $\lambda(f) = \eta(f^+) - \eta(f^-)$ is *linear*, and $\eta(f) = \lambda(|f|)$, (11) every set of elements of Σ having an upper bound has a least upper bound (and dually).

These results can be found in Kantorovitch, op. cit.

3. *Normal Subspaces and Decompositions.*—In proving our Mean Ergodic Theorem (Theorem 3), but not elsewhere, we shall want two further definitions, which seem to be new.

DEFINITION 2: *A subspace of a linear space satisfying* (P0)–(P3) *is called "normal" if and only if it contains* (a) *with any* f, *also* $|f|$, *and* (b) *with any positive* f, *all "parts" of* f (*i.e., all positive* x *with* $x < f$).

DEFINITION 3: *By a "direct decomposition" of* Σ, *is meant a choice of complementary normal subspaces—that is, of subspaces* S *and* T *such that* $S \frown T = 0$, $S + T = \Sigma$.

Remarks: (1) The normal subspaces of Σ correspond to its homomorphisms in just the same way that the normal subgroups of a group correspond to *its* homomorphisms, and (2) the decompositions of Σ correspond one–one to its representations as a direct union.

4. *Connections with Dependent Probabilities.*—Spaces (L) are connected with the theory of dependent probabilities by three fundamental definitions.

DEFINITION 4: *By a "distribution" is meant a positive element of* Σ *with norm one.*

DEFINITION 5: *By a "transition operator" on* Σ *is meant an additive operator which carries distributions into distributions.*

DEFINITION 6: *A transition operator* T *describing the dependence of the state of a system at time* t′ *on its state at a previous instant* t, *is called "independent" of an operator* U *relating the instants* t″ *and* t′ [$t'' > t'$], *if and only if the instants* t″ *and* t *are related by the transition operator* TU: $f \to (fT)U$.

As authorities for these definitions, we can cite the usual formulations of Bayes' Theorem, of the theory of Markoff chains, of Kolmogoroff's more general theory of "stochastic processes." Also, Fourier's theory of heat flow is expressed by transition operators: the invariance of $\lambda(f)$ is the gist of the first, and that of the set of $p \geqq 0$ of the second, law of thermodynamics. Finally, the flows of phase-space envisaged by Poincaré in his version of classical mechanics, induce automorphisms on the space (L)—and hence are transition operators in our sense, as well as "unitary operators on Hilbert space."

Conclusions: (1) The set Δ of all distribution functions is a closed convex subset of Σ, of diameter $\sup |(p - q)| \leqq 2$, (2) the distance $\eta(|p - q|)$ is the "stochastic distance" recently defined by Mazurkiewicz—it is *not* equivalent to the traditional notion of "convergence in probability," (3)

$|fT| \leqq |f|$, whence $\eta(fT) \leqq \eta(f)$ (T is of "modulus" unity, and a "contraction," and so uniformly continuous!).

5. *Hypothesis of Markoff.*—Now let Σ be any space (L), and T a fixed transition operator on Σ.

DEFINITION 7: *An* f *in* Σ *is called a "fixpoint" if and only if* $\mathrm{fT} = \mathrm{f}$. *A distribution which is a fixpoint is called "stable."*

Results: (1) The fixpoints are a closed linear subspace of Σ. Hence the stable distributions are a closed convex subset of Δ, and their number is either zero, or one (the "metrically transitive" case[5]), or infinity. All three cases are possible, but the second is the most interesting.

Hypothesis of Markoff (weakened): For some n, $d = \inf_{p \in \Delta} pT^n > 0$.

THEOREM 1: *If* T *satisfies Markoff's hypothesis, then there is a unique stable distribution* p_0. *Moreover the* pT^k *tend to* p_0 *uniformly, with the rapidity that the terms of a convergent geometrical progression tend to zero.*

Proof: First, $\eta(pT^n - qT^n) \leqq (1 - |d|)\eta(p - q)$ for any $p, q \in \Delta$. The conclusion now follows by a generalization to complete metric spaces (like Δ) of a simple argument due to Carl Neumann and often exploited by Picard, which is purely geometrical.

COROLLARY 1: $\mathrm{Sup\ pT}^n \leqq \mathrm{p} + \sum_{k=0}^{\infty} (\mathrm{pT}^{k+1} - \mathrm{pT}^k)$ *is finite, for any fixed* p. (Cf. §2, conclusion (11)).

COROLLARY 2: *Let* $\mathrm{T}_1, \ldots, \mathrm{T}_n$ *be any sequence of transition operators, and let* d_i *denote* $\mathrm{Inf}_{p \in \Delta} \mathrm{pT}_i$. *Then for all* p, q *in* Δ, $\eta(\mathrm{pT}_1 \ldots \mathrm{T}_n - \mathrm{qT}_1 \ldots \mathrm{T}_n) \leqq 2 \prod_{i=1}^{n} (1 - |\mathrm{d}_i|)$.

6. *Ergodic Hypothesis.*—Unless the conclusion of Corollary 1 holds, the means of the pT^k at best tend to 0. Hence we shall fix p, and make the

ERGODIC HYPOTHESIS: The pT^k have an upper bound. This is fulfilled if p is the integral of a bounded density-function, and T leaves measure invariant: the integral of the upper bound to the density function is an upper bound to the pT^k.

THEOREM 2: *The Ergodic Hypothesis implies the existence of at least one stable distribution.*

Proof: Form $h = \mathrm{Lim\ sup}_{k \to \infty} pT^k = \mathrm{Inf}_n \sup_{k \geqq n} pT^k$; evidently $hT = T$, $|h| \geqq 1$, and so $h > 0$. Hence $h/|h|$ is a stable distribution.

THEOREM 3 (mean ergodic theorem): *The Ergodic Hypothesis implies that the means* $\frac{1}{n} \sum_{k=0}^{n-1} \mathrm{pT}^k$ *converge* weakly, *in the sense that if* $\lambda(\mathrm{f})$ *is any linear functional, then the numerical means* $\phi_n(\mathrm{p}) = \lambda\left(\frac{1}{n} \sum_{k=0}^{n-1} \mathrm{pT}^k\right)$ *converge in the ordinary sense.*

Proof: By a generalization of a Lemma of Hahn (cf. §7), λ is the sum of its positive and negative parts. Again, by a simple Lemma of Banach

(op. cit., p. 54) each part is a constant multiple of a functional satisfying $0 \leqq \lambda(f) \leqq \eta(f)$ for all $f > 0$. Hence we need only consider this case. Again, if k is large, then $h = \lim \sup_{k\to\infty} pT^k$ contains an arbitrarily large part of pT^k, together with all transforms of this part; hence we can assume $p \leqq h$, where $hT = h$.

We shall make these assumptions, and in the proof, shall treat all "parts" f of h on the same footing. First, define $\bar{\phi}(f) = \lim \sup_{n\to\infty} \phi_n(f)$, $\underline{\phi}(f) = \lim \inf_{n\to\infty} \phi_n(f)$. Clearly $0 \leqq \underline{\phi}(f) \leqq \bar{\phi}(f) \leqq \eta(f)$; clearly also $\underline{\phi}(fT) = \underline{\phi}(f)$ and $\bar{\phi}(fT) = \bar{\phi}(f)$. The functionals $\underline{\phi}$ and $\bar{\phi}$ are monotone; they need *not* be linear, but $\underline{\phi}$ is convex while $\bar{\phi}$ is concave. Hence the functionals (we use a construction of F. Riesz)

$$(\bar{\alpha}f) = \sup \sum_i \bar{\phi}(f_i) \qquad \underline{\alpha}(f) = \inf \sum_i \underline{\phi}(f_i)$$

where the summations are with respect to all decompositions of f into (finite or countable; it makes no difference) parts f_i, are, respectively, the least linear functional $\geqq \bar{\phi}$, and the greatest linear functional $\leqq \underline{\phi}$. Moreover $\bar{\alpha}(fT) = \bar{\alpha}(f)$ and $\underline{\alpha}(fT) = \underline{\alpha}(f)$; the functionals are invariant.

Now since $0 \leqq \underline{\alpha} \leqq \underline{\phi} \leqq \bar{\phi} \leqq \bar{\alpha} \leqq \eta$, and $h \geqq f$, in order to conclude $\underline{\phi}(f) = \bar{\phi}(f) = \mathrm{Lim}_{n\to\infty} \phi(f)$, we need only show that $\underline{\alpha}(f) = \bar{\alpha}(f)$, whence, since $\bar{\alpha} - \underline{\alpha}$ is non-negative and linear, we need only show $\bar{\alpha}(h) \leqq \lambda(h) \leqq \underline{\alpha}(h)$. By duality, we need only show $\bar{\alpha}(h) \leqq \lambda(h)$. This is just what we shall prove.

7. *Extension of a Lemma of Hahn.*—For it, we shall need an extension of a Lemma of Hahn.[6] Let $\lambda(x)$ be any linear functional on Σ, let Λ^+ denote the set of $u > 0$ such that $0 < x \leqq u$ implies $\lambda(x) > 0$, and define Λ^- dually. Further, let Λ^0 denote the set of $u > 0$ such that $0 < x \leqq u$ implies $\lambda(x) = 0$.

LEMMA: *Σ is decomposed into three components: a component Λ^+ on which $x > 0$ implies $\lambda(x) > 0$, a component Λ^0 on which $\lambda(x) = 0$, and a component Λ^- on which $x > 0$ implies $\lambda(x) < 0$.*

COROLLARY 1: *Any linear functional can be resolved into its positive part and its negative part.*

8. *Completion of Proof.*—Choose $\epsilon > 0$, and denote by g_n the component of h (cf. §7) on which $\phi_n(x) - \bar{\alpha}(x) + \epsilon\eta(x)$ is non-negative. Then irrespective of ϵ,

LEMMA: *The join* u *of the* g_n *is* h.

Proof: Consider $r = h - u$; evidently for all n, $r \leqq h - g_n$. Hence $\bar{\phi}(x) \leqq \sup \phi_n(x) \leqq \bar{\alpha}(x) - \epsilon\eta(x)$ for all $x \leqq r$, and so $\bar{\alpha}(r) = \sup \Sigma\bar{\phi}(x_i) \leqq \bar{\alpha}(r) - \epsilon\eta(r)$, whence $\eta(r) = 0$ and $r = 0$.

Now let h_n denote the part of g_n not in $g_1 \cup \ldots \cup g_{n-1}$; the h_n are the components of a direct decomposition of h. Choose M so large that if h^* denotes $h - \sum_{k=1}^{m} h_k$, then $|h^*| < \epsilon$. We shall show that for all $N > M$,

$$\text{(E)} \quad N\lambda(h) \geqq N\bar{\alpha}(h) - N\epsilon\eta(h) - N\epsilon - M\bar{\alpha}(h)$$

from which, dividing through by N, and letting $N \to \infty$, we will get $\lambda(h) \geqq \bar{\alpha}(h) - \epsilon\eta(h) - \epsilon$. Now letting $\epsilon \to 0$, the proof is complete.

The proof of (E) reproduces the combinatorial essence of G. D. Birkhoff's proof of the Strong Ergodic Theorem.[2] We rely on the fact that the "components" of h form a Boolean algebra, and may be treated like sets.

[1] L. Kantorovitch, "Lineare halbgeordnete Raume," *Math. Sbornik*, **2**, 121–68 (1937). Cf. also H. Freudenthal, "Teilweise geordnete Moduln," *Proc. Akad. Wet. Amsterdam*, **39**, 641–51 (1936).

[2] J. von Neumann, "Proof of the Quasi-Ergodic Hypothesis," these PROCEEDINGS, **18**, 70–82 (1932). Our method is that used by G. D. Birkhoff for his stronger result; cf. "Proof of a Recurrence Theorem for Strongly Transitive Systems, and Proof of the Ergodic Theorem," these PROCEEDINGS, **17**, 650–60 (1931).

[3] In the sense of the author's "On the combination of subalgebras," *Proc. Camb. Phil. Soc.*, **29**, 441–64 (1933). Synonyms are "Verband" (Fr. Klein) and "structure" (O. Ore). We shall use the notation $f \cup g$ for sup (f, g) and $f \cap g$ for inf (f, g).

[4] S. Banach, "Théorie des opérations linéaires," Warsaw, 1933. By general consent, the "B-spaces" of Banach, op. cit., are called Banach spaces; they are complete, metric, linear spaces.

[5] In the sense of G. D. Birkhoff and Paul Smith, "Structure Analysis of Surface Transformation," *Jour. Math.*, **7**, 365 (1928).

[6] The author is much indebted to J. von Neumann for suggesting that this lemma could be generalized. He is also indebted to S. Ulam for many conversations on the whole subject.

ON SEMI-GROUPS OF TRANSFORMATIONS IN HILBERT SPACE

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1. Let E be a normed complete linear vector space, in other words a space (B) in the terminology of Banach. Let $x \epsilon E$ and let $T_\alpha(x)$ be a linear transformation on E to E defined for every $\alpha > 0$. If

$$T_\alpha(T_\beta(x)) = T_{\alpha+\beta}(x), \tag{1}$$

we say that $\{T_\alpha(x)\}$ forms a *semi-group*.

Assuming in addition that

$$\| T_\alpha(x) \| \leqq \| x \|, \tag{2}$$

I have investigated some of the properties of these transformations.[1] Continuing this study, I have found the case in which E is a Hilbert space and $T_\alpha(x)$ is a self-adjoint, positive definite transformation of particular

interest. In this case there exists a representation of $T_\alpha(x)$ analogous to that found by M. H. Stone for unitary transformations forming a group.[2]

2. The main result is the following theorem.

Let $T_\alpha(x)$, $\alpha > 0$, be a family of self-adjoint, positive definite transformations on $\mathfrak{H}$ to $\mathfrak{H}$ satisfying (1) *and* (2). *Then there exists a self-adjoint transformation $A(x)$, positive definite but not necessarily bounded, with its resolution of the identity $E(\lambda)$, such that*

$$(T_\alpha(x), x) = \int_0^\infty e^{-\alpha\lambda} d_\lambda(E(\lambda)x, x). \tag{3}$$

It follows in particular that for a fixed x, $(T_\alpha(x), x)$ is an analytic function of α, holomorphic for $\Re(\alpha) > 0$ and continuous for $\Re(\alpha) \geqq 0$. This would seem to be the reason why the proof can be carried through without any assumptions regarding the continuity or measurability of $(T_\alpha(x), x)$.

A detailed proof will be published elsewhere, but the following outline of the argument will probably be found sufficient to enable the interested reader to fill in the omissions. The basic observation is that

$$\Delta_h^n(T_\alpha(x), x) = \sum_{k=0}^{n} (-1)^k \binom{n}{k} (T_{\alpha + kh}(x), x) \geqq 0$$

for every $\alpha \geqq 0$, $h > 0$, $n \geqq 0$. Hence $(T_\alpha(x), x)$ is completely monotone in $0 \leqq \alpha < \infty$ and by the Bernstein-Widder theorem[3]

$$(T_\alpha(x), x) = \int_0^\infty e^{-\alpha\lambda} dV(\lambda; x), \tag{4}$$

where $V(\lambda; x)$ is a never decreasing function of λ and $V(0; x) = 0$. By (2) $0 \leqq (T_\alpha(x), x) \leqq (x, x)$ so that $V(\lambda; x) \leqq (x, x)$.

The real inversion formulas for the Laplace integral show that $V(\lambda; x)$ is a linear functional in $(T_\alpha(x), x)$. The latter being bilinear in x, we conclude the existence of a bilinear functional $V(\lambda; x, y)$ such that

$$(T_\alpha(x), y) = \int_0^\infty e^{-\alpha\lambda} dV(\lambda; x, y), \tag{5}$$

and $V(\lambda; x, x) = V(\lambda; x)$. Further

$$|V(\lambda; x, y)|^2 \leqq (x, x)(y, y),$$

whence it follows that $V(\lambda; x, y) = (E(\lambda)x, y)$. The transformations $E(\lambda)x$ are evidently self-adjoint. Replacing x by $E(\mu)x$ in (5) and putting $\alpha = 0$, we easily conclude that $E(\lambda)x$ is a resolution of the identity. We then define the corresponding self-adjoint transformation $A(x)$ in the usual manner by

$$(A(x), y) = \int_0^\infty \lambda \, d_\lambda(E(\lambda)x, y),$$

the domain of $A(x)$ being that subset of $\mathfrak{H}$ for which

$$\int_0^\infty \lambda^2 d_\lambda(E(\lambda)x, x) < \infty.$$

That $A(x)$ is positive definite follows from the fact that $E(\lambda) = 0$ for $\lambda \leqq 0$. Incidentally we observe that it is permitted to interpret $A(x)$ as a determination of $-\log T_1(x)$.

3. Among the various transformations forming semi-groups we select the Poisson integral for the half-plane, i.e.,

$$P_\alpha(f) = \frac{\alpha}{\pi}\int_{-\infty}^{\infty} \frac{f(t+u)}{u^2+\alpha^2}\,du. \tag{6}$$

If $f(t) \in L_2(-\infty, \infty)$ so does $P_\alpha(f)$ and conditions (1) and (2) are satisfied. Moreover, $P_\alpha(f)$ is a self-adjoint, positive definite transformation. A straightforward calculation gives

$$(P_\alpha(f), f) = \int_{-\infty}^{\infty} e^{-\alpha|\lambda|}\,|F(\lambda)|^2\,d\lambda,$$

where $F(\lambda)$ is the Fourier transform of $f(t)$. Putting

$$D_\lambda(f) = \frac{1}{\pi}\int_{-\infty}^{\infty} \frac{\sin \lambda u}{u} f(t+u)\,du \tag{7}$$

for $\lambda > 0$, $D_\lambda(f) = 0$ for $\lambda \leqq 0$, and utilizing the relation

$$(D_\lambda(f), f) = (D_\lambda(f), D_\lambda(f)) = \int_{-\lambda}^{\lambda} |F(u)|^2\,du,\ \lambda > 0,$$

we find

$$E(\lambda)f = D_\lambda(f),\ A(f) = \tilde{f}', \tag{8}$$

where $\tilde{g}(t)$ is the conjugate function of $g(t)$.

[1] E. Hille, "Notes on Linear Transformations. I," *Trans. Amer. Math. Soc.*, **39**, 131–153 (1936).

[2] M. H. Stone, "Linear Transformations in Hilbert Space," these PROCEEDINGS, **16**, 172–175 (1930), and "On One-Parameter Unitary Groups in Hilbert Space," *Ann. Math.* (2) **33**, 643–648 (1932). See also J. von Neumann, "Über einen Satz von M. H. Stone," *Ann. Math., Ibid.*, 567–573, and F. Riesz, "Über Satze von Stone und Bochner," *Acta Szeged*, **6**, 184–198 (1933).

[3] S. Bernstein, "Sur les fonctions absolument monotones," *Acta Math.*, **52**, 1–66 (1929). D. V. Widder, "Necessary and Sufficient Conditions for the Representation of a Function as a Laplace Integral," *Trans. Amer. Math. Soc.*, **33**, 851–892 (1931).

[4] See loc. cit., note[1] for the properties of $P_\alpha(f)$ and $D_\lambda(f)$.

RESIDUATED LATTICES

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1. *Introduction.*—We summarize here our investigations of a lattice $\Sigma: a, b, \ldots, z$ over which a multiplication or a residuation is defined. (Ward 1, Dilworth 1.) We denote division, union and cross-cut by $x \supset y$, (x, y), $[x, y]$. Σ is closed with respect to union (cross-cut) if any set of elements have a union (cross-cut). The unit and null elements i and n are defined by $i \supset x$, $x \supset n$, every $x.a$ covers b (Birkhoff 1) if $a \supset b$, $a \neq b$ and $a \supset x \supset b$ implies $x = a$ or $x = b$. Elements covered by i are called divisor-free. A sub-lattice Λ is dense over Σ if Λ contains l, m and $l \supset x \supset m$ imply Λ contains x. An element a is a node if either $x \supset a$ or $a \supset x$, every x. Any involution of Σ interchanging union and cross-cut is called a negation. An element a is idempotent relative to a binary operation $x \circ y$ if $a \circ a = a$. Two properties P and Q which Σ may possess are completely independent if there exist instances of lattices in which both P and Q hold, neither holds, P holds but not Q, Q holds but not P.

2. *Residuations and Multiplications.*—Assume Σ contains i. A well defined binary operation $x:y$ is called a residuation over Σ provided that

R 1. a:b *lies in* Σ *whenever* a, b *lie in* Σ.
R 2. a:b = i *if and only if* a $\supset$ b.
R 3. a $\supset$ b *implies* a:c $\supset$ b:c *and* c:b $\supset$ c:a.
R 4. (a:b):c = (a:c):b.
R 5. [a, b]:c = [a:c, b:c] *and* c:(a, b) = [c:a, c:b].

This residual has the formal properties of the residual in polynomial ideal theory. (Ward 1, Dilworth 1.)

THEOREM. *A residuated lattice closed with respect to cross-cut is also closed with respect to a well-defined multiplication* x·y *satisfying the following conditions:*

M 1. a·b *lies in* Σ *whenever* a, b *lie in* Σ.
M 2. (a·b)·c = a·(b·c).
M 3. a·b = b·a.
M 4. a·i = a.
M 5. a·(b, c) = (a·b, a·c).

If a multiplication over Σ satisfies M 1–M 5 and M 6: *The product of the unions of any two sets of elements of* Σ *is the union of the products of all pairs of elements of the sets,* then a residuation exists satisfying R 1–R 5. (Ward 1.) The relationship between the two operations is as follows.

$a \supset (a:b)\cdot b$; if $a \supset x\cdot b$ then $a:b \supset x$.
$(a\cdot b):a \supset b$; if $x:a \supset b$ then $x \supset a\cdot b$.

Both operations may be dualized.

THEOREM. *The Dedekind modular condition and the existence of a residual are completely independent properties of a lattice. The existence of a residual and the existence of a negation are completely independent.*

3. *Conditions for Residuation.*

THEOREM. *Every distributive lattice which is closed with respect to union can be residuated in at least one way.* (Ward 2.)

THEOREM. *Every Boolean algebra can be residuated in only one way.*

The residual in this case is $a:b = a \vee b'$. (Dilworth 1.) A lattice is said to be complemented (Birkhoff 2) if it contains i and n and for every element a an element a' such that $(a, a') = i$, $[a, a'] = n$.

THEOREM. *The only complemented lattices which can be residuated are Boolean algebras.*

COROLLARY. *No non-trivial projective geometry* (Birkhoff 2) *can be residuated.*

THEOREM. *The free modular lattice of order twenty-eight cannot be residuated.*

THEOREM. *Every lattice in which only one divisor free element exists can be residuated in at least one way.*

THEOREM. *A lattice built up out of a set of residuated lattices connected into a chain by nodes can be residuated.*

THEOREM. *A direct product of residuated lattices can be residuated; conversely if a residuated lattice can be expressed as a direct product, each of its factors can be residuated.*

THEOREM. *A necessary condition that a residuated lattice in which an ascending chain condition holds* (Ore 1) *can be residuated is that every Boolean algebra generated by a finite number of divisor free elements be dense over the lattice.*

4. *Noether Lattices.*—We propose here the name "Noether lattice" for any residuated modular lattice in which both the (ascending) chain condition holds and

D 1. *For any two elements* a, b *of* Σ, *there exist exponents* r *and* s *such that* $a\cdot b \supset [a^r, b^s]$.

For the ideal theory terminology used here see van der Waerden 1.

THEOREM. *In a Noether lattice, every irreducible is primary. Conversely, if in a residuated modular lattice with chain condition every irreducible is primary, then condition* D 1 *holds.*

THEOREM. *The three decomposition theorems and the uniqueness theorems of* E. *Noether for the ideals of a commutative ring in which the chain condition holds are all valid in an abstract Noether lattice.*

THEOREM. *Conditon* D 1 *is completely independent both of the modular condition, the distributive condition and the chain condition.*

THEOREM. *A necessary and sufficient condition that a finite residuated modular lattice be a Noether lattice is that* $a \cdot b = [a, b]$ *for all idempotent elements* a, b *of the lattice.*

THEOREM. *A sufficient condition that a residuated modular lattice in which the ascending chain condition holds be a Noether lattice is*

M 7. $a \cdot [b, c] = [a \cdot b, a \cdot c]$.

The resulting lattice need not be distributive.

5. *Distributive Residuated Lattices.*—Consider a lattice in which one or more of the following conditions hold:

D 2. *If* $a \supset b$, *there exists at least one element* q *such that* $a \cdot q = b$.
R 6. $(a:b, b:a) = i$.
R 7. $a:[b, c] = (a:b, a:c)$.
R 8. $(b, c):a = (b:a, c:a)$.

THEOREM. *Every lattice closed with respect to union in which* D 2 *holds can be residuated, and is distributive.*

THEOREM. *If* Σ *is a residuated lattice, any one of* R 6, R 7, R 8 *implies* Σ *is distributive.* R 6 *and* D 2 *implies* R 7 *and* R 8. R 8 *implies* R 7.

We call a residuated lattice satisfying D 2 and R 6 *semi-arithmetical.* The properties of such lattices are similar to the instance in Ward 2 where multiplication is cross-cut.

6. *Residuated Group Lattices—Dual Operations.*—On assuming Σ is a semi-group and D_2, we may pass to the group Γ of quotients a/b. We have made Γ into a residuated lattice having properties R 1–R 8. However the lattice has no unit element.

The existence of a dual residuation and multiplication is completely independent of the existence of the initial residuation and multiplication. An interesting case arises in a residuated lattice containing n if the correspondence $a \rightarrow n:a$ is a negation. The lattice is then distributive, and multiplication and its dual are also distributive with respect to one another.

Proofs of these results will be published elsewhere.

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